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THE ROLE OF THE PLANT DISEASE SURVEY IN FORECASTING PLANT DISEASES*

PAUL R. MILLER AND MURIEL J. O'BRIEN

(Accepted for publication, October 20, 1954)

INTRODUCTION

Before very long the increase in world population will bring about serious pressure in food resources. In fact, the danger point has nearly been reached in some regions. To feed an ever-increasing population is one of the major agricultural tasks confronting the world today. To some extent this task can be accomplished by bringing more land into production, but the amount of additional land which could be used for agricultural purposes is probably not sufficient to meet this great and increasing demand. Even with more acres under cultivation, we must make use of every means to improve the efficiency and lessen the risks of agricultural production as it exists now.

Among the most important means of lessening risks in crop production are efficient prevention and control of plant diseases. However, the actual application of a specific control measure is not enough by itself. It is important to watch the occurrence of diseases so that prompt action can be taken to prevent losses, and to study critically the factors involved in epidemic outbreaks. This is the two-fold work of the Crop Plant Disease Forecasting Project as conducted by the United States Department of Agriculture.

DISCUSSION

Early Work :

In the following account, discussion of the work of the Plant Disease Survey is limited to those aspects that have contributed to the evolution of the forecasting project. The essential elements were evident in the very beginning of the Survey's activities, almost forty years ago at the time of World War 1. Knowledge about plant disease occurrence in general was inadequate then, and one of the reasons for the establishment of the Survey was the realization that lack of information about potentially serious hazards to agiculture might have grave consequences, especially in wartime. Therefore, *The Plant Disease Reporter* (then called *The Plant Disease Bulletin*) was founded to publish regular, frequent, current reports on disease development on major crops. These reports were sent in by the Survey's collaborators, that is, State plant pathologists who gave voluntary assistance. Surveys to determine the distribution of diseases affecting specific crops, or of single important or potentially important diseases, were also carried out and the results published. Later the Survey began to conduct special field studies in cooperation with several States (Archer, 1926, 1928, 1929). A staff member identified the diseases

*The paper was kindly furnished by the authors on invitation

occurring on the crops grown in the State, determined their distribution, estimated the amount of loss they caused, and finally placed specimens of each disease in the Mycological Collections of the former Bureau of Plant Industry for permanent reference.

Middle Period :

Concentration on special problems became the dominant note in the second phase of the Survey's work. Two pressures characteristic of the times determined this course : one was the demand for specific information on certain crop diseases ; the other was the strong emphasis on economics in the 1930's.

Several surveys within this period can now be recognized as forerunners to the establishment of the Warning Service in its present form, and served at the time as examples of the advantages to be gained from forecasting. Studies of a series of reports of bacterial wilt (*Bacterium stewartii* E. F. Sm.) of sweet corn (*i.e.*, maize, *Zea mays* L.), together with weather records, demonstrated that the occurrence of the disease fluctuated according to the temperature of the preceding winter. A simple precise method of forecasting severity in the coming season for any given locality was worked out, depending on the sums of the average temperatures for the preceding winter months (December, January, February). The method is still in use, with some modifications, wherever sweet corn is grown (Stevens, 1945 ; Stevens and Haenseler, 1941). The basis for predicting the occurrence of tobacco blue mold (*Peronospora tabacina* Adam) was also determined. Earliness of appearance, rapidity of spread, and severity of the disease were shown to be correlated with January temperatures above normal in the South Atlantic Coast States (Miller, 1937 ; Miller and O'Brien, 1949). The Warning Service's experience of the past few years has confirmed the accuracy of these observations. Another achievement of this period was the discovery of the reasons for some hitherto puzzling discrepancies in the distribution of the rust diseases (*Gymnosporangium* spp.) of apple (Miller, 1932, 1934, 1939).

Emergency Plant Disease Prevention Programme :

These investigations can, in retrospect, be seen as leading up to our present Warning Service and the epidemiological research programme. The results obtained were decidedly helpful in meeting the demands upon plant pathological knowledge during World War II, when protection of crops again became a major concern in national defense. The Emergency Plant Disease Prevention Programme was set up with a superior field staff of experienced plant pathologists to ensure crop safety, primarily by preventing malicious demage from enemy action. The findings of this fundamental defense task are still unpublished, but some other activities, although incidental to the real purpose of the Emergency Programme, contributed a great deal of the experience that makes the Crop Plant Disease Forecasting Project a success. The reports on the appearance and progress of potato late blight constituted an effective "warning service" during an unprecedented epidemic in the Southern States in 1943-44. The field men also located and reported incipient outbreaks of diseases to

make sure that chemical control materials, which were scarce during the war, reached the places where they were most needed in time to be used effectively (Miller and Wood, 1947).

Throughout this period forecasting was being increasingly recognized as an effective aid in control and, indeed for some diseases, as an indispensable part of control measures. For some diseases reporting services were giving good results. Among them was a potato late blight warning service conducted by the Mississippi Valley Plant Pathologist's Committee during the war (Melhus, 1945).

At the end of the war, the accumulated evidence from the various sources was so strong that grower, industrial, and scientific organizations formally stated their interest in the formation of a national Warning Service, with a programme to include fundamental research on the epidemiology of certain downy mildew diseases and the establishment of regional forecasting facilities. The particularly destructive epidemic of tomato late blight in 1946 crystallized the demand for immediate organization of this forecasting programme. Many unexplained or only partly understood circumstances of the outbreak made it evident that the warning service, necessary for immediate protection, would be too superficial to get at the real causes of these downy mildew epidemics and would have to be backed by research to determine the basic factors needed for accurate forecasts. Moreover, it was obvious that both the warning service and the research should be not merely local but should apply to wide areas to obtain most benefit.

Current Project:

The work of the Forecasting Project can best be defined by its title: "Establishment of facilities for forecasting the development of crop plant diseases by (*a*) intensive field work, in co-operation with State Agricultural Experiment Stations and others interested, to obtain accurate data on the occurrence and distribution of economic crop diseases and to search for new and threatening diseases or dangerous outbreaks of established diseases, and (*b*) epidemiological research on the factors involved in these outbreaks".

Warning Service:

In general, the Warning Service is assigned the task of securing accurate data on occurrence and distribution of three downy mildew diseases, late blight of potato and tomato (*Phytophthora infestans* (Mont.) D By.), blue mold of tobacco (*Peronospora tabacina* Adam), and downy mildew of cucurbits (*Pseudoperonospora cubensis* (Berk. & Curt.) Rostow.), which are the primary concern of the Forecasting Project at present. These well-known diseases were chosen because their mode of attack, dissemination, and survival have, in part, some similar aspects which would permit forecasting their probable spread. Moreover, the crops they attack are important economic crops which are grown over extensive areas. The crop production is seasonal, moving northward from the earlier-producing southern regions as the growing season progresses. This continous production affords an

opportunity to watch the advance of the diseases in their northward spread. Also, another factor operating in the choice of these diseases, is that they can be economically controlled by the organic fungicides now on the market. The Department of Agriculture and the State Agricultural Experiment Stations are jointly responsible for its operation, and the fungicide and control equipment industries and canning and other grower organizations also contribute to its effectiveness. It operates through plant pathologists in each of the 48 States of the United States and 10 Provinces of Canada.

The chart in Figure 1 shows the organization of the Warning Service, its relationship with cooperating pathologists and agencies, and the manner in which it works. Figure 2 shows the connection between the Warning Service and the Survey proper.

The appearance and spread of each disease included in the Service are reported promptly to the Plant Disease Survey. The Survey compiles these reports into a warning letter sent twice weekly during the growing season to all key pathologists and other authorised recipients. These reports on occurrence and severity of these diseases in places with an earlier growing season, together with previous experience and the weather forecasts, provide the key pathologists with enough information to judge rather accurately whether or not the local crops are likely to be affected, and, if so, how soon and how seriously. Thus, when necessary, they can warn growers in advance that they should be ready to protect their plants, or, on the other hand, in periods of low disease activity time, energy, and money are saved by knowing that control measures will not be required. Control chemicals and equipment are available where and when most needed because agricultural chemical associations and farm equipment companies also receive the warning letters.

Research :

In October 1947 an intensive research programme was started. It is concerned with occurrence and distribution of the organisms ; the effect of environment, particularly microclimate, on their establishment and survival ; the existance of strains, some perhaps more virulent than others ; and the life history and development of these fungi, especially factors influencing germination and infection. From the scientific viewpoint, the results obtained during the past six years have contributed a great deal to fundamental knowledge both of the fungi and of the factors that affect development of disease, and have laid the groundwork for the initiation of some experiments in forecasting disease occurrence on a regional basis. Practically speaking, the accuracy of the local forecasts has been much improved by making use of these findings.

The effect of environment on disease development is being explored from several angles. One approach has been analysis of weather data and potato late blight records for the past half century in both the Atlantic Seaboard States and the North Central States regions.

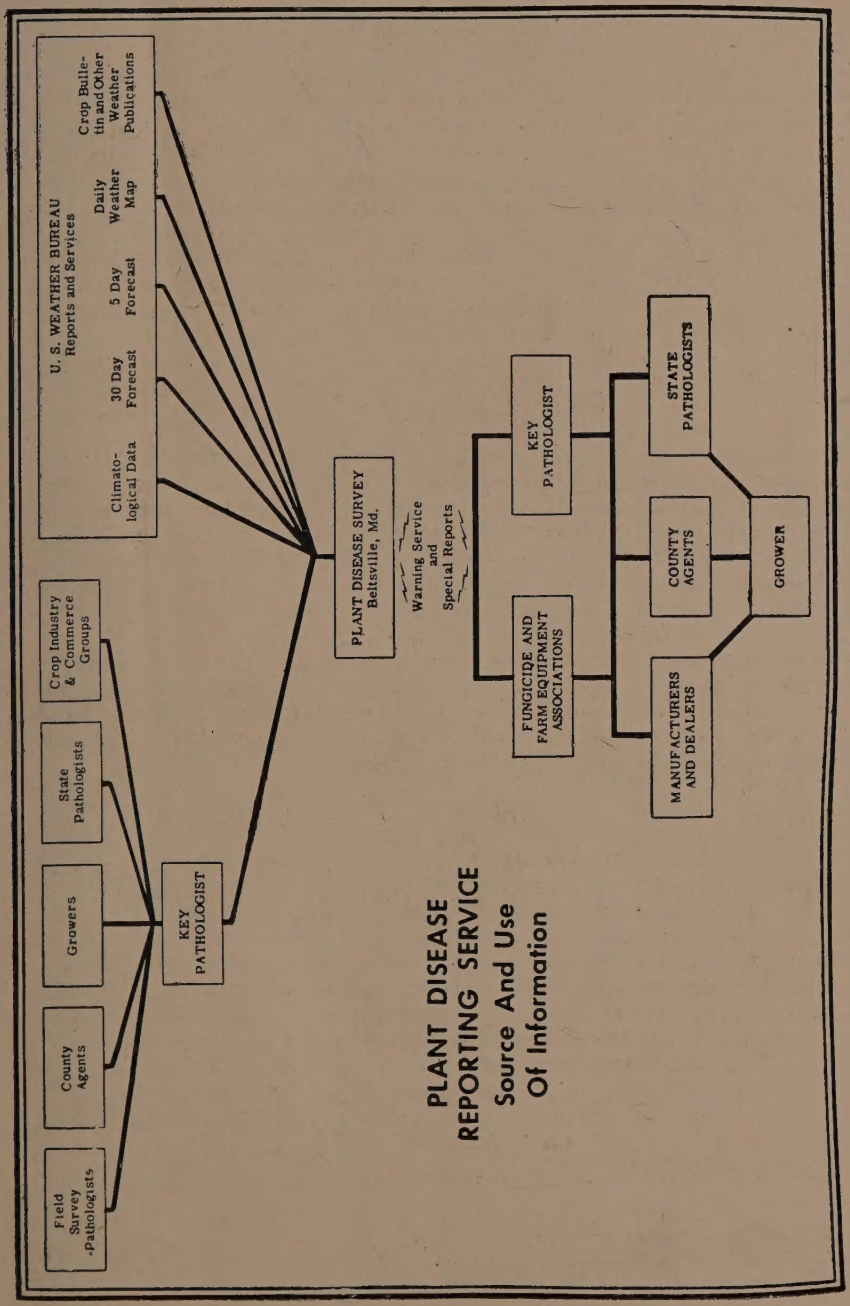
PLANT DISEASE
REPORTING SERVICE
Source And Use
Of Information
Field Survey -Pathologists
County Agents
Growers
State Pathologists
Crop Industry & Commerce Groups
KEY PATHOLOGIST
U. S. WEATHER BUREAU
Reports and Services
Climato-logical Data
30 Day Forecast
5 Day Forecast
Daily Weather Map
Crop Bulle-tin and Other Weather Publications
PLANT DISEASE SURVEY
Beltsville, Md.
Warning Service and Special Reports
KEY PATHOLOGIST
FUNGICIDE AND FARM EQUIPMENT ASSOCIATIONS
MANUFACTURERS AND DEALERS
COUNTY AGENTS
STATE PATHOLOGISTS
GROWER

FIG. 1

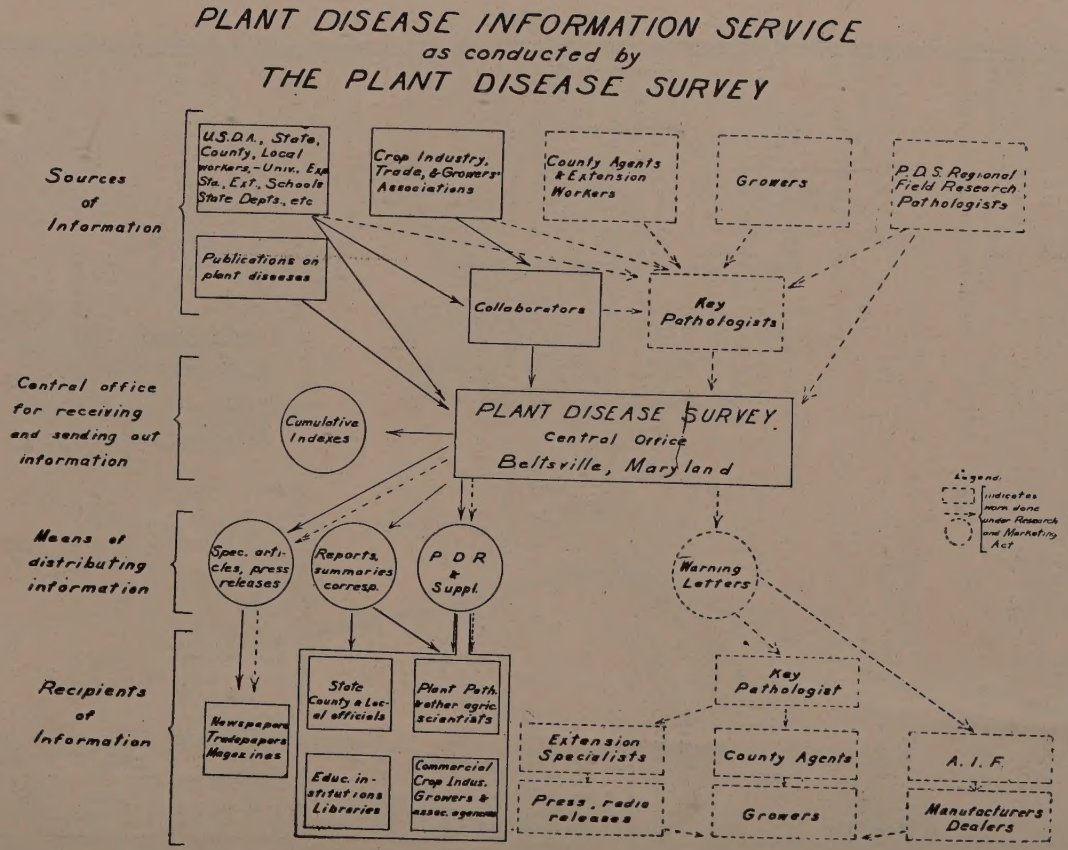
PLANT DISEASE INFORMATION SERVICE
as conducted by
THE PLANT DISEASE SURVEY
Sources of Information
U.S.D.A., State, County, Local workers, - Univ., Exp Sta., Ext., Schools State Depts., etc
Crop Industry, Trade, & Growers' Associations
County Agents & Extension Workers
Growers
P.D.S. Regional Field Research Pathologists
Publications on plant diseases
Collaborators
Key Pathologists
Control office for receiving and sending out information
Cumulative Indexes
PLANT DISEASE SURVEY
Central Office
Beltsville, Maryland
Means of distributing information
Spec. articles, press releases
Reports, summaries corresp.
P D R & Suppl.
Warning Letters
Legend.
indicates work done under Research and Marketing Act
Recipients of Information
Newspapers Tradepapers Magazines
State County & Local officials
Plant Path. & other agric. scientists
Educ. institutions Libraries
Commercial Crop Indus. Growers & assoc. agencies
Key Pathologist
Extension Specialists
County Agents
A. I. F.
Press, radio releases
Growers
Manufacturers Dealers

FIG. 2

In the Atlantic Coast region some very satisfactory schemes for predicting occurrence of late blight have resulted from these comparisons. Briefly they depend on observed association of late blight appearance and spread with the combination of temperature in a definite range and rainfall above a certain minimum existing together for a period of time long enough to allow infection and further development.

In practice the current season weather data are compared with the standards of favourable weather for late blight occurrence derived from the long-time records. The method was first worked out by H. T. Cook for Virginia and South Carolina in the South Atlantic area (Cook, 1947, 1948, 1949). Study of Connecticut records showed that the method was essentially applicable to the Northeastern Atlantic Coast area also (Hyre and Horsfall, 1951), although greater accuracy in this area was obtained with some changes in the manner of using the basic weather data to obtain a more precise definition of "critical" temperatures and rainfall (Hyre, 1954).

In the North Central region, on the other hand, rainfall and temperature are not sufficient to indicate favourable conditions for blight development, according to study of data for Indiana, Iowa, and Wisconsin (Wallin and Waggoner, 1949; Wallin and Samson, 1953; Wallin *et al.*, 1953). Atmospheric humidity seems to be the important factor in this region, and it has been shown that the hygrograph provides a more complete account of humidity conditions in relation to late blight occurrence than do rainfall data alone, because this machine records the humidities associated not only with rainfall, but with dew deposition, fog, and saturated atmosphere as well (Wallin *et al.*, 1953).

Experimental investigation has produced significant results. The discovery that viable sporangia of the late blight fungus are adrift in the air at least until noon extends the known period during each day in which infection can take place (Wallin and Waggoner, 1950). Viable sporangia were found on potato and tomato foliage during afternoons when temperatures were between 80° and 90° F, and during the following evenings as well, proving that the late blight fungus can withstand lower humidities and higher temperatures than was previously supposed. Also, viable sporangia are produced within six hours on potato foliage during the hottest part of the day, a period usually considered adverse to sporulation (Wallin, 1953). Spread of late blight was followed from an inoculated plant to the edge of infection. Spread was gradual, from plant to plant, rather than by discontinuous jumps from the centre to the periphery (Wallin and Waggoner, 1950).

Movement of sporangia in the air, against air currents (crosswind), with air currents (downwind), and vertically, from a point source was measured. The greatest distance from the source of sporangia that blight appeared was on the downwind side and toward obstructions to air movement. The percentage of leaflets with blight lesions showed a rapid initial decrease and became progressively smaller as distance from the source of inoculum increased. Thus, spores

from nearby sources are more important in the spread of late blight than spores carried from a distance (Waggoner, 1952).

In the microclimatic studies no biologically significant differences were revealed between temperature and humidity as recorded at one-foot or plant-level site and the normal U. S. Weather Bureau's instrument shelter height of five feet above the soil. This is important because it shows that weather data obtained at the usual instrument heights can be used in the development of forecasting systems (Waggoner, 1952).

In an investigation into strain differences in *Phytophthora infestans* Waggoner and Wallin (1952) demonstrated the existence of a tomato race and a potato race, which were about equally pathogenic to potato but differed somewhat in development on tomato. These races were most often isolated from their specific hosts but each could also be isolated from the other host. Hyre (1949) suggested that there is only one tomato race of *P. infestans* and that the potato strain affects tomatoes in a minor way. The existence of different races of the fungus is important in breeding resistant varieties.

Lima bean downy mildew (*Phytophthora phaseoli* Thaxt.) is often severe in the Central Atlantic coast region where the crop is an important one. The physiology and life history of the fungus are being studied to establish bases for forecasting. Conditions necessary for its growth were found to include the presence of free water for the germination of sporangia, a temperature of 20° C for maximum radial growth *in vitro*, and a temperature of 20° with 95 to 100 per cent relative humidity for maintaining sporangia at maximum viability. Zoospore germination and germ tube elongation are in direct ratio to increase in temperature up to 25° (Hyre and Cox, 1953).

A successful method for germinating the oospores of *Peronospora tabacina*, the tobacco blue mold organism, is an outstanding achievement of the research programme. Germination occured by the production of sessile zoosporangia attached directly to the oospores. This type of germination had not been observed in the genus *Peronospora* before. The discovery is a distinct contribution to knowledge of infection phenomena in the downy mildews (Person and Lucas, 1953; and Lucas and Person, 1954).

In some of the studies the techniques and equipment to be used constituted an important part of the problem. For example, a special sampling method was developed to obtain a correct estimate of the number of viable sporangia of *Phytophthora infestans* in the air at various times of the day (Wallin and Waggoner, 1950). Formulae were worked out for measuring the spatial distribution of sporangia in various directions from a point source (Waggoner, 1952). Wallin and Waggoner's (1952) investigations on strains required a precise separation of the effects attributable to each single factor among the various factors influencing disease. From thorough review of previous work on the subject of races in fungi they defined four types of races according to the particular one of the four factors—host, pathogen, environment, and time—correlated with significantly different amounts of disease. The definitions and mathematical expression will be useful in all similar studies.

A record of dew deposition was often required. Since no instrument for the automatic recording of dew existed, one was invented. The new instrument records the onset and duration of dew deposition and is simple in construction and operation. Its use will add greatly to our knowledge concerning the relation of dew to secondary infection (Wallin and Polhemus, 1954).

The information gained from this fundamental research on infection phenomena, life cycles, and relation of weather to the occurrence and spread of these fungi has enabled us to attempt experimental regional forecasts for late blight. These experimental forecasts are similar to the weather forecasts in that data are interpreted and predictions sent out from a central station instead of locally. Obviously this procedure, if successful, would save considerable time in relaying predictions, which might often be the deciding factor between good and poor control. These forecasts have been made in the North Central region for the past several years. They are based on temperature—humidity relations of the organism and have proven to be extremely accurate, blight usually occuring as predicted. Recently, the main concern in formulating the forecasts has been to point out the minimum temperature and humidity levels under which the blight fungus might survive and develop in the field. A great deal of the spread of blight in this region takes place under these minimum conditions.

Conclusion and Summary

Miller and O'Brien (1952) have brought the whole subject of plant disease forecasting together in one review article. One section is devoted to warning services conducted throughout the world, not only for late blight but for other diseases as well. Another summarizes research contributing to forecasting. Miller (1950) discussed the work of the Plant Disease Survey in relation to forecasting and to plant pathology in general.

From its earliest establishment the Plant Disease Survey, by publishing regular, frequent, current reports on disease development in major crops, began the work that led up to the present day forecasting. Concentration on thorough studies of the incidence and importance of certain crop diseases was the second stage in evolution. Finally, the current forecasting project was directly due to the evident success of the Emegency Plant Disease Prevention Programme conducted by the Plant Disease Survey during World War II and to the concern aroused by the severe tomato late blight epidemic of 1946.

The fundamental research accomplished from the initiation of the forecasting project in 1947 to the present time has greatly increased our knowledge on the environmental requirements and life histories of certain downy mildew fungi. This knowledge has formed the basis for experimental regional forecasts which have attained great accuracy.

In conclusion, we should say that in the reorganization of the United States Department of Agriculture, the name Plant Disease Survey was discarded. Instead of being the Plant Disease Survey, of

the Division of Mycology and Disease Survey, we are now Plant Disease Epidemics, of the Plant Disease Epidemics and Identification Section.

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Beltsville, Md., U.S.A.

REFERENCES

Archer, W. A. (1926) Plant diseases in 1926—Unnumbered mimeographed publication, University of Missouri, Columbia, Missouri.

———————. (1928) Plant diseases in Iowa in 1927. *Plant. Dis. Reptr. Suppl.* **58**.

———————. (1929) Plant diseases in West Virginia in 1928. *Plant Dis. Reptr. Suppl.* **72**.

Cook, H. T. (1947) Our method for forecasting tomato late blight. *Plant Dis. Reptr.* **31** : 245-249.

———————. (1948) Forecasting late blight for the Charleston, South Carolina, area from Norfolk, Virginia. *Plant Dis. Reptr. Suppl.* **178** : 217-219.

———————. (1949) Forecasting late blight epiphytotics of potatoes and tomatoes. *Jour. Agr. Res.* **78** : 545-563.

Hyre, R. A. (1949) A study of the occurrence of the races of *Phytophthora infestans* in northeastern United States. *Plant Dis. Reptr.* **33** : 177-179.

———————. (1954) Progress in forecasting late blight of potato and tomato. *Plant Dis. Reptr.* **38** : 245-253.

———and R. S. Cox. (1953) Factors affecting the viability and growth of *Phytophthora phaseoli. Phytopathology* **43** : 419-425.

———and James G. Horsfall. (1951) Forecasting potato late blight in Connecticut. *Plant Dis. Reptr.* **35** : 423-431.

Lucas, G. B. and L. H. Person. (1954) Factors influencing oospore germination in *Peronospora tabacina. Plant Dis. Reptr.* **38** : 243-244.

Melhus, I. E. (1945) Late blight forecasting service. *Phytopathology.* **35** : 463-479.

Miller, Paul R. (1932) Apple rust surveys. *Plant Dis. Reptr.* **16** ; 158-168.

———————. (1934) A method of estimating the percentage of infection of apple leaves with observations on the relative susceptibility of species and varieties of *Malus* and *Juniperus*. *Plant Dis. Reptr.* 18: 159-163.

———————. (1937) January temperatures in relation to the distribution and severity of downy mildew of tobacco. *Plant Dis. Reptr.* 21: 260-266.

———————. (1939) Incidence and importance of quince rust on apple as affected by environmental and developmental factors. *Plant Dis. Reptr.* 23: 80-82.

———————. (1950) The place of the Plant Disease Survey in plant pathological investigation. *Plant Dis. Reptr. Suppl.* 195: 471-482.

———————and Muriel O'Brien. (1949) January temperatures in relation to the distribution and severity of downy mildew of tobacco. II. A review of the past nineteen years. *Plant Dis. Reptr.* 33: 418-425.

———————and ———————. (1952) Plant disease forecasting. *The Botanical Review* 18: 547-601.

———————and Jessie I. Wood. (1947) An evaluation of certain phases of the Emergency Plant Disease Prevention Project. *Plant Dis. Reptr. Suppl.* 167.

Person, L. H. and G. B. Lucas. (1953) Oospore germination in *Peronospora tabacina*. *Phytopathology* 43: 701-702.

Stevens, Neil E. (1945) Research and plant disease surveys. *Plant Dis. Reptr. Suppl.* 152: 6-12.

———————and C. M. Haenseler. (1941) Incidence of bacterial wilt of sweet corn. 1935-1940: Forecasts and performance. *Plant Dis. Reptr.* 25: 152-157.

Waggoner, Paul E. (1952) Distribution of potato late blight around inoculum sources. *Phytopathology* 42: 323-328.

———————and J. R. Wallin. (1952) Variation in pathogenicity among isolates of *Phytophthora infestans* on tomato and potato. *Phytopatholgy* 42: 645-648.

Wallin, J. R. (1953) The production and survival of sporangia of *Phytophthora infestans* on tomato and potato plants in the field. *Phytopathology* 43: 505-508.

———————and Dale N. Polhemus. (1954) A dew recorder. *Science* 119: 294-295.

———————and R. W. Samson. (1953) The relation of cumulative amount and frequency of rainfall and mean temperature to late blight in Indiana. *Amer. Potato Jour.* **30** : 262-270.

———, E. K. Wade, and H. M. Darling. (1953) The relation of weekly mean temperatures and cumulative rainfall to late blight epiphytotics in Wisconsin. *Amer. Potato Jour.* **30** : 231-242.

———————and Paul E. Waggoner. (1949) The influence of weekly cumulative rainfall and temperature on potato late blight epiphytotics in Iowa. *Plant Dis. Reptr.* **33** : 210-218.

———————— and ————————. (1950) The influence of climate on the development and spread of *Phytophthora infestans* in artificially inoculated potato plots. *Plant Dis. Reptr. Suppl.* **190** : 19-33.

SOME NEW BACTERIAL DISEASES OF PLANTS

M. K. PATEL, Y. S. KULKARNI & G. W. DHANDE

(Accepted for publication, August 8, 1954)

In a previous paper[1], a preliminary account of three bacterial diseases incited by new species of *Xanthomonas* was described by the authors, and an extended study of which is presented below :—

1. Bacterial leaf-spot of teak (*Tectona grandis* Linn.). Teak popularly known as *Sag* is a large deciduous tree grown for its timber in peninsular India and in parts of Burma, Java and Sumatra. In Bombay State, the teak timber is of good quality and compares favourably with that imported from other places. Bark is used as an astringent and also for the preparation of dyes and oil extraction. Oil from nuts is used as a hair tonic. Leaves are used all over the teak growing districts as materials for packing and thatching roofs. A bacterial leaf-spot causing severe infection and deformation was noticed on plants at Ambernath (Bombay State) in October, 1950.

SYMPTOMS

The pathogen incites numerous, angular, water-soaked translucent spots measuring 0.5 to 1 mm. and delimited by veins. Viewed against light when fresh, infection spots appear golden yellow in colour and, on drying, they turn brown to deep-brown in colour and raised on the upper surface due to encrustation of dried bacterial ooze. Several such spots often coalesce and form large lesions which cause premature defoliation of the trees and retard growth of young shoots. Infection on petiole or stem has not been noticed. The incubation period is about 4 to 7 days.

MORPHOLOGY

The pathogen can be isolated easily by the usual poured plate method. The organism is a short rod, mostly single, rarely in chains of two or more, and with no involution forms. It measures $2.1 \times 1.08\mu$. It is motile by a polar flagellum, gram negative, non-spore former, capsulated and not acid fast. It can be stained readily with common dyes.

CULTURAL AND PHYSIOLOGICAL CHARACTERS

On potato dextrose agar plates, colonies are circular, without striations, glistening, pulvinate, diameter 1.5 cm. in 10 days, Empire yellow (R)* ; On nutrient agar plates, growth poor, colonies round with entire margin, flat, measuring 1 cm. in 7 days, Empire yellow (R).

1. Patel, M. K., Y. S. Kulkarni and G. W. Dhande (1952). Current Science 21 : 345-346.

*Ridgway's colour standard is followed.

On nutrieut dextrose agar plates, colonies are round with entire margin, convex, measuring 2 cm. in 7 days, Primuline yellow (R); on potato cylinders, growth slow, not shining, covering the entire surface in about 4 days; Empire yellow (R), colour of the potato cylinder remaining unchanged.

The organism liquefies gelatin and digests starch and casein; litmus reduced; nitrate not reduced to nitrite; ammonia and hydrogen sulphide produced; M.R. and [illegible] negative; Loeffler's blood serum completely liquefied in 4 da[illegible]; sodium chloride tolerant upto 2 per cent. Thermal death point about 51°C.

The organism grows well in several synthetic media separately containing 1 per cent dextrose, sucrose, lactose, galactose, maltose, glycerol, mannitol, dextrin, arabinose and raffinose with the production of acid but no gas. It fails to ferment salicin. Grows well in acetic and citric but not in tartaric and oxalic acids.

Host Range

The organism was specific in infection behaviour and could only infect *Tectona grandis* but not *Pisum sativum* L., *Medicago sativa* L., *Vigna catjang* Walp., *Xanthium strumarium* L., *Cicer arietinum* L., *Ricinus communis* L., *Citrus sinensis* Osbeck., *Cajanus cajan* Milsp., *Sesbania aegyptiaca* Pers., *Ipomoea muricata* R., and Sch., *Cyamopsis tetragonoloba* Taub., *Solanum melongena* L., *Desmodium diffusum* DC., *Cassia tora* L., *Trigonella phoenum-graecum* L., *Stizolobium deeringianum* Bort., *Arachis hypogaea* L., *Desmodium gangeticum* DC., *Triticum sativum* L., *Clerodendron phlomoides* Linn., *Capsicum annuum* L., *Begonia* sp. and *Lathyrus sativus* L.

Taxonomy and Nomenclature

The organism described above has not previously been reported from India or any other place and is a typical species of *Xanthomonas*. Study of the cultural characters and host range indicates that it is an undescribed species. The name *Xanthomonas melhusii*, named in honour of Dr. I. E. Melhus, formerly of Iowa State College, U. S. A. is proposed for its accommodation.

Technical Description

Xanthomonas melhusii Patel, Kulkarni and Dhande sp. nov., short rods, mostly single or rarely in chains of two or more, measuring $2.1 \times 1.8\mu$, motile by a pollar flagellum, gram negative, non-spore former, capsulated; not acid fast, aerobe, stains readily with common dyes; slow growth on potato cylinders; on potato dextrose agar slants, growth copious; on nutrient dextrose agar plates, colonies are circular without striations and of Primuline yellow colour; acid but no gas from dextrose, lactose, maltose, sucrose; no growth in salicin as well as in oxalic and tartaric acids; grows well in citric and acetic acids; gelatin liquefied; starch and casein hydrolysed; no growth in Uschinsky's and Cohn's solutions, slight growth in Fermi's; good growth

on Endo's agar, colonies measuring 1.1. cm. in 10 days without metallic sheen around the colonies; good growth in Simmon's citrate and Patel's agar, fair growth in Koser's uric acid medium; litmus reduced; nitrite not produced from nitrate; ammonia from neutral beef broth +1% KNO_3 and hydrogen sulphide produced; indol. M.R. and V.P. tests negative; Loeffler's solidified blood serum completely liquefied within 15 days; sodium chloride tolerant upto 2 per cent; Optimum temperature for growth between 25° and 30°C., thermal death point near 51°C.

Pathongenic on *Tectona grandis* producing leaf-spots. Found first at Ambernath by Y. S. Kulkarni in November 1950 and later at several other places in Bombay State.

2. Bacterial leaf-spot of *Erythrina indica* L.—*Erythrina indica*, popularly known as "Pangara" is a moderate sized, quick growing tree often used as hedge, support as well as shelter in the betelvine gardens all over India. In Bengal, it is used in the black pepper gardens for the same purpose. Bark of the tree and tender leaves are used by some along with currey and leaves are used as cattle fodder in some places. Wood of *Pangara* is durable, light and used for preparing boxes, tags and trays. A bacterial leaf-spot was noticed on this plant at Patna (Bihar), Poona and Dharwar in November, 1951.

Symptoms

On leaves, the disease appears as specks which are numerous, angular, water-soaked becoming brown and are usually surrounded by pale yellowish halo, measuring 1.5 to 2 mm. On the under surface of the leaves, the spots are raised in the centre and flat on the upper surface. The entire leaflet becomes chlorotic due to numerous spots which measure 0.75 to 1.0 mm. Veins and vein-lets are also infected. Infection is found more on the leaf-edges although the entire surface of leaves at one or other places is found to be affected.

Morphology

The pathogen is a short rod with rounded ends, mostly single or rarely in chains of two or more with no involution forms. The organism from a week old culture on potato dextrose agar measures $1.6 \times 1.2\mu$. It is motile by a polar flagellum, gram negative, non-spore former, capsulated not acid fast, and stains readily with common dyes.

Cultural and Physiological Characters

In nutrient agar plates, growth is poor, flat, colonies measuring 1.0 cm. in 7 days, colour Primuline yellow (R); on nutrient dextrose agar plates, colonies are round with entire margin, convex, measuring 2 cm. in 7 days, colour Pinard yellow (R); on nutrient dextrose agar plates, colonies are round with entire margins, convex, measuring 2 cm. in 7 days, colour Primuline yellow (R). On potato dextrose agar plates, colonies are round, pulvinate, 1.6 cm. in diameter after 10 days, striations not present, colour lemon crome (R).

The organism liquefies gelatin and is able to digest starch and casein, reduces litmus, not reducing nitrate to nitrites ; ammonia and hydrogen sulphide produced ; M.R. and V.P. tests negative ; Loeffler's solidified blood serum slowly liquefied ; sodium chloride tolerant upto 2 per cent. Thermal death point is about 51°C.

The organism grows well in several synthetic carbohydrate media separately containing 1 per cent dextrose, maltose, lactose, sucrose, glycerol, raffinose and dextrin with the production of acid but no gas. It fails to grow in salicin. Grows well in citric but not in tartaric, oxalic and acetic acids.

Host Range

The organism was host specific and could infect only *Erythrina indica* but not *Tectona grandis*, *Pisum sativum* L., *Medicago sativa* L., *Vigna catjang* Walp., *Xanthium strumarium* L., *Cicer arietinum* L., *Ricinus communis* L., *Citrus sinensis* Osbeck., *Cajanus cajan* Milsp., *Sesbania aegyptiaca* Pers., *Ipomoea muricata* R. and Sch., *Cyamopsis tetragonoloba* Taub., *Solanum melongena* L., *Desmodium diffusum* DC., *Cassia tora* L., *Trigonella phoenum-graecum* L., *Stizolobium deeringianum* Bort., *Arachis hypogaea* L., *Desmodium gangeticum* DC.. *Triticum sativum* L., *Clerodendron phlomoides* Linn., *Capsicum annuum* L., *Begonia* sp. and *Lathyrus sativus* L.

Taxonomy and Nomenclature

Since the organism described is new to science and its morphological, cultural and biochemical characters do not resemble any member of the genus *Xanthomonas*, it is proposed to name it *Xanthomonas erythrinae* sp. nov. whose technical description is given below :

Technical Description

Xanthomonas erythrinae Patel, Kulkarni and Thirumalachar sp. nov. short rods, mostly single, rarely in chains of two or more, measuring 1.6×1.1μ; motile by a single polar flagellum; gram negative; non-spore former ; capsulated ; not acid fast ; stains readily with common dyes ; on potato dextrose agar, colonies are pulvinate, round, glistening, butyrous, with no striations and entire margins, 1.6 cm. diameter in 10 days, colour lemon crome (R) ; acid but no gas from dextrose, lactose, maltose, sucrose, glycerol, raffinose and dextrin ; grows well in citric acid but not in tartaric, oxalic and acetic acids. It fails to ferment salicin ; gelatin liquefied ; starch and casein hydrolysed ; no growth in Uschinsky's and Cohn's but slight growth in Fermi's solutions ; good growth in Endo's agar, colonies measuring 1.1 cm. after 10 days with no metallic sheen around the colonies ; good growth in Simmons's citrate and Patel's agars ; poor growth in Koser's uric acid medium ; litmus reduced ; nitrites not produced from nitrates, ammonia and hydrogen sulphide produced ; indol, M.R. and V.P. tests negative ; Loeffler's blood serum slowly liquefied ; sodium chloride tolerant upto 2 per cent ; optimum temperature for growth 28°C.; thermal death point near 51°C.

Pathogenic on *Erythrina indica* producing brown, angular, water-soaked specks on leaves, causing chlorosis and deformities in severe cases. Found at Patna (Bihar) by M. J. Thirumalachar, November 1952, Poona, Dharwar and several other places in Bombay State.

3. A bacterial leaf spot of *Trichodesma zeylanicum* Br.- *Trichodesma zeylanicum* is a common herb found in the Deccan Peninsula and Ceylon where its leaves are often used in making poultice. A bacterial leaf-spot was noticed on it at Chitalenagar (District Sholapur) in November, 1951.

Symptoms

The disease appears all over the leaves as small, round, water-soaked spots which soon enlarge in size and measure 0.5 to 1 mm. in diameter. Spots when close, coalesce to form irregular lesions which are jet black and raised a little on the upper surface, the corresponding area on the lower surface becoming depressed. Veins are also seen infected. Bacterial ooze in the form of shining flakes is found on the spots on the lower surface of the leaves. In severe cases due to numerous spots coalescing, part of the leaf or even the whole leaf dries up and is finally shed.

Morphology

The pathogen which can easily be isolated by usual poured plate method is a short rod, mostly single or rarely in chains of two or more and with no involution forms. It measures $2.2 \times 1.3\ \mu$. It is motile by a single polar flagellum, gram negative, non-spore former. capsulated and not acid fast. It can be readily stained with common dyes.

Cultural and Physiological Characters

On potato dextrose agar plates, colonies are pulvinate, circular with entire margin, measuring 1.9 cm. in diameter after 10 days with striations starting from the centre to the periphery ; colour amber yellow (R) ; in nutrient agar plates, growth is poor ; colonies are round, flat, glistening with entire margin, measuring 0.9 cm. in 7 days; colour Empire yellow (R). In nutrient dextrose agar plates, colonies are round with entire margin, convex, measuring 1.5 cm. in 7 days, Mustard yellow (R). On potato cylinders, growth copious, shining, covering the entire surface in about 4 days, colour Primuline yellow (R), colour of the potato cylinder remaining unchanged.

The organism liquefies gelatin and is able to digest starch, casein ; litmus is reduced but not nitrate to nitrite ; ammonia and hydrogen sulphide produced; M.R. and V.P. tests negative ; Loeffler's blood serum completely liquefied after 8 days ; sodium chloride tolerant upto 2 per cent ; thermal death point near 50°C.

The organism grows well in several synthetic media separately containing 1 per cent dextrose, lactose, sucrose, galactose with the production of acid but no gas. It fails to grow in salicin.

FIG. 1

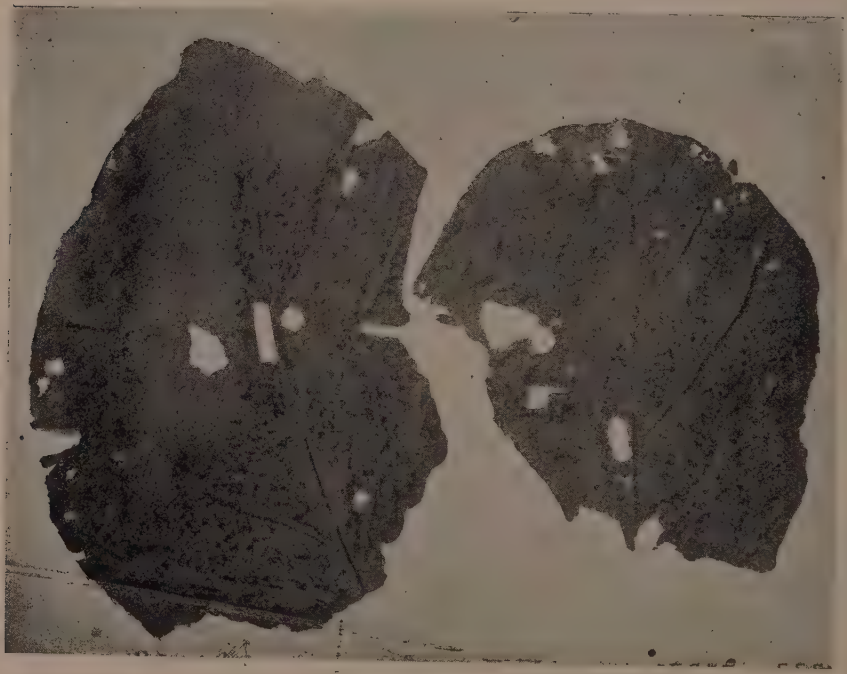

FIG. 2

Host Range

All the hosts previously used for inoculation of Teak and *Pangara* organisms were inoculated including *Trichodesma zeylanicum*. The organism was host specific every time as to infect *T. zeylanicum* only and repeated trials to infect the rest of the plants failed.

Taxonomy and Nomenclature

Since the organism under study in its morphological, cultural and physiological reactions does not tally with those of the genus *Xanthomonas* described by Dowson[2], it is proposed to name it *Xanthomonas trichodesmae* sp. nov.

Technical Description

Xanthomonas trichodesmae Patel and Kulkarni sp. nov.—Short rod ; mostly single or rarely in chains of two or more measuring $2.2 \times 1.3\ \mu$; motile by a single polar flagellum , Gram negative ; non-spore former ; capsulated ; not acid fast ; aerobe ; stains readily with common dyes; copious, shining growth on potato cylinders ; on potato dextrose agar plate, growth good, amber yellow (R) ; colonies on nutrient dextrose agar, are circular with entire margin, flat, measuring 1.5 cm. in 5 days, colour amber yellow (R), acid but no gas from dextrose, sucrose, lactose, galactose ; no growth in salicin ; gelatin liquefied ; starch and casein hydrolysed ; no growth in Uschinsky's, Cohn's and Fermi's solutions, no growth in Koser's uric acid medium, slight growth in Simmons's citrate and Patel's agars, good growth in Endo's agar but the metallic sheen absent ; slight growth in Czapeck's agar ; nitrates not reduced to nitrites, ammonia and hydrogen sulphide produced ; indol, M.R. and V.P. tests negative; Loeffler's blood serum completely liquefied within 15 days ; sodium chloride tolerant upto 2 per cent ; optimum temperature for growth between 25° and 30°C ; thermal death point 50°C.

Pathogenic on *Trichodesma zeylanicum* Br. inciting leaf-spots and causing deformities and defoliation when severe. First noticed at Chitalenagar (Dist. Sholapur) by Y. S. Kulkarni and later at several places in Bombay State.

Summary

Morphology, cultural characters and inoculation experiments with three bacterial phytopathogens viz. *Xanthomonas melhusii* nov. sp. *X. erythrinae* and *X. trichodesmae* sp. nov. on *Tectona grandis*, *Erythrina indica* and *Trichodesma zeylanicum* respectively are recorded and detailed account of each is given.

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2. Dowson, W. J. (1949). Manual of bacterial plant diseases. Adam & Charles Black, London.

Explanation of plate

Plate I

Fig. 1. Leaves of *Tectona grandis* showing numerous, angular, water-soaked spots often coalescing. Leaf edges are also infected.

Fig. 2. Leaves of *Erythrina indica* showing numerous, angular, water-soaked spots surrounded by halo. Veins and leaf-edges are also infected.

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LEAF SPOT DISEASE OF TURMERIC (*CURCUMA LONGA* L.) CAUSED BY *COLLETOTRICHUM CAPSICI* (Syd.) BUTL. & BISBY

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(Accepted for publication August 19, 1954)

Turmeric (*Curcuma longa*) is an important commercial crop cultivated in various parts of the world (chiefly in the tropics). In India it is grown in several states extending from Assam and Bihar in the north down to Madras in the south. It is grown under irrigation in well-drained fertile soil. Outside India, it has spread wherever Indians have migrated, as in Malaya, East Indies, West Indies, Kenya, Nyasaland, Mauritius and Seychelles. It is reported to be unknown in the wild state in India and probably is a native of Cochin China (Sampson 1936). It is cultivated for the sake of its rhizomes which are used as a condiment, an ingredient in many of the medicinal preparations of the indigenous systems of medicine and as a source of yellow dye.

A leaf spot disease of this crop has been prevalent in Coimbatore (Madras State), Cuddappah, Kurnool, Guntur, Krishna and Godavari districts (Andhra State) for over 3 decades. The first record of the disease was by McRae in 1917 from Coimbatore District. The specimens were sent to Sydow for identification of the pathogen, which he named as *Vermicularia curcumae*. Sundararaman (1926) carried out comparative studies of several isolates of *Vermicularia* and came to the conclusion that the isolates obtained from chillies, turmeric, cabbage, knolkhol and cauliflower belonged to the same species, viz., *V. capsici* Syd. Butler and Bisby (1931) revised *V. curcumae* Syd. as *Colletotrichum curcumae*, but did not merge it in *C. capsici*, though they have stated that Sundararaman considered *V. curcumae* to be the same as *V. capsici*. Ramakrishnan (1947) made detailed studies of *C. capsici*, *C. curcumae* and *C. indicum* Dast, and stated that all these should be merged in one species. Though the disease had been observed years ago, a comprehensive description of the disease and the methods of controlling the same have not been published as yet. Since the time of the first report of the occurrence of the disease, it has been recorded every year in varying intensity in different parts of peninsular India. Severe outbreaks have been recorded from Erode and Bhavani (Coimbatore District) and from Cuddappah, Guntur, Krishna and Godavari districts.

Symptoms of the disease : The disease manifests itself on the leaves in the form of elliptic or oblong spots, variable in size. In the initial stages, they are small but very soon many of them increase in size and may measure one and a half to two inches in length and about an inch to an inch and a half across. Two or more spots may coalesce developing into irregular patches often involving a

major portion of the leaf, which eventually dries up. Each individual spot has a characteristic appearance. The centre is greyish white and thin with numerous black, dot-like acervuli on both surfaces. These are arranged in concentric rings. Beyond the greyish white portion is a brown margin all round the spot. On this also the acervuli may be seen. Outside this is an indefinite yellowish region forming a halo round the spot. The spots, though visible on both surfaces, are more marked on the upper surface in the fresh leaves. But on drying, they are equally conspicuous on both surfaces. Invariably, the lesions are on the leaf blade. But sometimes stromata or acervuli may be seen on the leaf sheaths also. When the incidence of the disease is heavy, most of the leaves dry up and the field presents a parched-up appearance. The central region of the spot may become papery and easily torn. Owing to the destruction of the chlorophyll-bearing leaf area, the production of rhizomes is reduced sometimes to less than 50%. Stromatoid bodies are formed even on the scales of the rhizomes.

The Pathogen: The acervuli are located intra-epidermally. The hyphae are septate and hyaline in the initial stages passing between and inside the cells of the mesophyll. Later, pale brownish hyphae accumulate inside the eqidermal cells and form the basis of stroma development. The stromata are made up of light to dark brown pseudoparenchymatous cells. The outer wall of the epidermis is ruptured and the setae and conidiophores are exposed. Setae are formed from all over the acervulus and are not confined to the margin. Crescent-shaped conidia are borne on the conidiophores.

The stromata are variable in size, ranging from 60 to 120 μ in diameter. The setae are brown, septate and tapering. The length of the setae is variable and ranges from 50 to 145μ. It has been reported by earlier workers that the setae in *Colletotrichum* are subject to wide variation in frequency per acervulus and in size (Ramakrishnan 1941). The measurements given by Sundararaman (1925) are in general agreement with these, though the upper limits of the diameter of the stroma (90 μ) and setae (90 μ) are lower than those obtained by the writer. These structures exhibit wide variation in size and, hence, their measurements are not to be relied upon for specific differentiation.

The conidia are crescent-shaped, hyaline and one-celled, measuring $25\times3\mu$ ($17-31\times3-4$). These are in agreement with those of *C. capsici* on typical specimens of chillies. A conspicuous vacuole is present in the centre of the conidium. But, as it grows old, the contents become more granular or highly vacuolated and the solitary vacuole is not evident. The conidial masses are of a pink, though individual spores are hyaline. The spores are embedded in a gelatinous substance and on drying they are all held together. But when the mass is floated in drops of water, the spores readily separate. The conidia germinate readily producing germ tubes from either or both ends. A septum is also developed dividing the cell into two compartments. Olive-brown, round or irregular thick- walled appressoria are formed at the ends of the germ tubes or from the tip of the spore itself.

Cultural characters : The fungus was brought into pure culture, and its growth on different media was studied. These characters are in agreement with those of *C. capsici* recorded earlier (Ramakrishnan 1941). On oat agar, the isolate exhibited good growth producing pale, olive-grey aerial mycelium and numerous buff-pink acervuli in the course of a week. The acervulus has a black stromatic base on which masses of conidia are produced to give a buff-pink colour to the fructification. On french bean agar, the aerial growth is a mixture of white and grey. Black stromata and pink acervuli are developed in large numbers in six to seven days. When the isolates are maintained in agar media for several generations, there is a reduction in the quantity of aerial mycelium produced. The surface of the slant becomes studded with numerous stromata and acervuli. Chlamydospores are formed in large numbers, especially at the junction of the surface of the slant and the sides of the test tube.

Pathogenicity : Inoculations were carried out on *Curcuma longa* and some other hosts on which allied or related species of the pathogen have been observed. Healthy plants, specially grown for the purpose, were sprayed with the suspension of the conidia and kept covered by bell jars or placed inside glazed cages for 3 days to provide favourable conditions for infection. Control plants were also maintained under identical conditions. The results are given below :—

TABLE I

Results of inoculation experiments

Host Plant	Part inoculated	Number inoculated	Number infected	Incubation period
Curcuma longa	Leaves	20	16	8-10 days
Capsicum annuum	Fruits	20	14	6-10 „
Cicer arietinum	Stem & pods	20	14	5- 8 „
Aristolochia bracteata	Leaves	20	15	5- 8 „
Withania somnifera	Fruits	15	10	7- 8 „
Gossypium herbaceum	Seedlings	15	0	0
Solanum melongena	Young fruits	10	7	8-10 „
Brassica oleracea var. *capitata*	Leaves	10	6	8-10 „

The controls remained healthy throughout. Lesions developed on the inoculated leaves of *C. longa*, *A. bracteata* and *B. oleracea* var. *capitata*. In the course of 10 days acervuli were also evident on the lesions. Sunken water-soaked lesions developed on the fruits of *C. annuum*, *W. somnifera* and *S. melongena* and in course of time the entire fruit was involved. Numerous acervuli developed on the affected fruits. In *Cicer arietinum* the pathogen caused complete destruction of the plant. The pods and seeds also were affected. Concentric lines of acervuli formed on the fruits. The seedlings of cotton were not affected. But, when the fungus was cultured on sterilised cotton seeds for five generations, successful infection of the seedlings was obtained.

Identity of the pathogen : It has been mentioned earlier that the identity of the fungus has undergone a number of changes. Since the fungus was originally described from infected turmeric leaves, the specific name was determined on host specificity. Butler and Bisby (1931) revised the generic name, but retained the species. Sundararaman (1926) has definitely stated that the species is similar to the one occurring on *Capsicum annuum*, viz., *C. capsici*. The results of cultural and pathogenicity studies of *C. curcumae* had led Ramakrishnan (1947) also to conclude that it was identical with *C. capsici* and had to be merged with it. The morphological characters of the fungus and its host range described in this paper bring out the fact that there is no justification for maintaining the isolate on turmeric as a distinct species. It is to be designated as *C. capsici*.

Control of the disease : The disease becomes evident when the crop is about 4 months old, *i.e.*, in the months of August—September. The incidence is, however, favourably influenced by high and continuous humidity of the atmosphere. Heavy infection has been frequently experienced in Cauvery Valley in Madras State and in Krishna District of Andhra State. Infection is mainly air-borne. Sundararaman (1925) has indicated that the pathogen may be seed-borne, being carried as dormant stromata on the scales of rhizomes. But the method of preservation of the seed rhizomes is not conducive to this fungus remaining dormant for 4—5 months, the interval between harvest and planting. The seed rhizomes are buried in the soil while planting and there is no evidence of infection spreading from the soil to the leaves. Furthermore, the pathogen is of common occurrence on a variety of hosts, both cultivated plants and weeds growing mixed with or in the neighbourhood of the turmeric crop. Therefore, there is no dearth of infective material. All these indicate that air-borne infection of the foliage occurs.

Air-borne infection can be controlled by protective spraying of the foliage with a fungicide. Experiments, carried out at Bhavani, have clearly shown that one per cent Bordeaux mixture sprayed on the crop before the commencement of the disease keeps down infection. The spray application is made early in August. In some years, two applications may be necessary. The addition of a wetting agent (crude oil emulsion) is advantageous. Both the surfaces of the

leaves have to be covered. The results obtained in two seasons are given below :—

TABLE II

Results of spraying the crop with Bordeaux mixture

Treatment	Yield of green rhizomes in pounds from 5 cents	
	First season	Second season
Sprayed	2379	1903
Unsprayed	1369	1146

There was a significant reduction in the incidence of leaf spot on the sprayed plants and this had resulted in an increased yield of rhizomes. Further trials were conducted in Tiruchirappalli district. A block of turmeric crop, an acre in extent, was sprayed with Bordeaux mixture. The incidence of the leaf spot was kept down (Sundararaman 1925). Based on these results, large-scale spraying of the crop is now being recommended for adoption by the farmers. Besides Bordeaux mixture, other proprietary copper fungicides are also under trial. Some of them have been found to be satisfactory.

SUMMARY

A leaf spot disease of turmeric caused by *Colletotrichum capsici* has been prevalent for a number of years in Madras and Andhra States. The symptoms of the disease are described. The isolate of the pathogen from turmeric passes on to the leaves of cabbage, and *Aristolochia bracteata*, seedlings of *Gossypium herbaceum* and *Cicer arietinum* and fruits of *Solanum melongena*, *Withania somnifera* and chillies. Preventive spraying with Bordeaux mixture on the foliage of turmeric keeps the disease under control.

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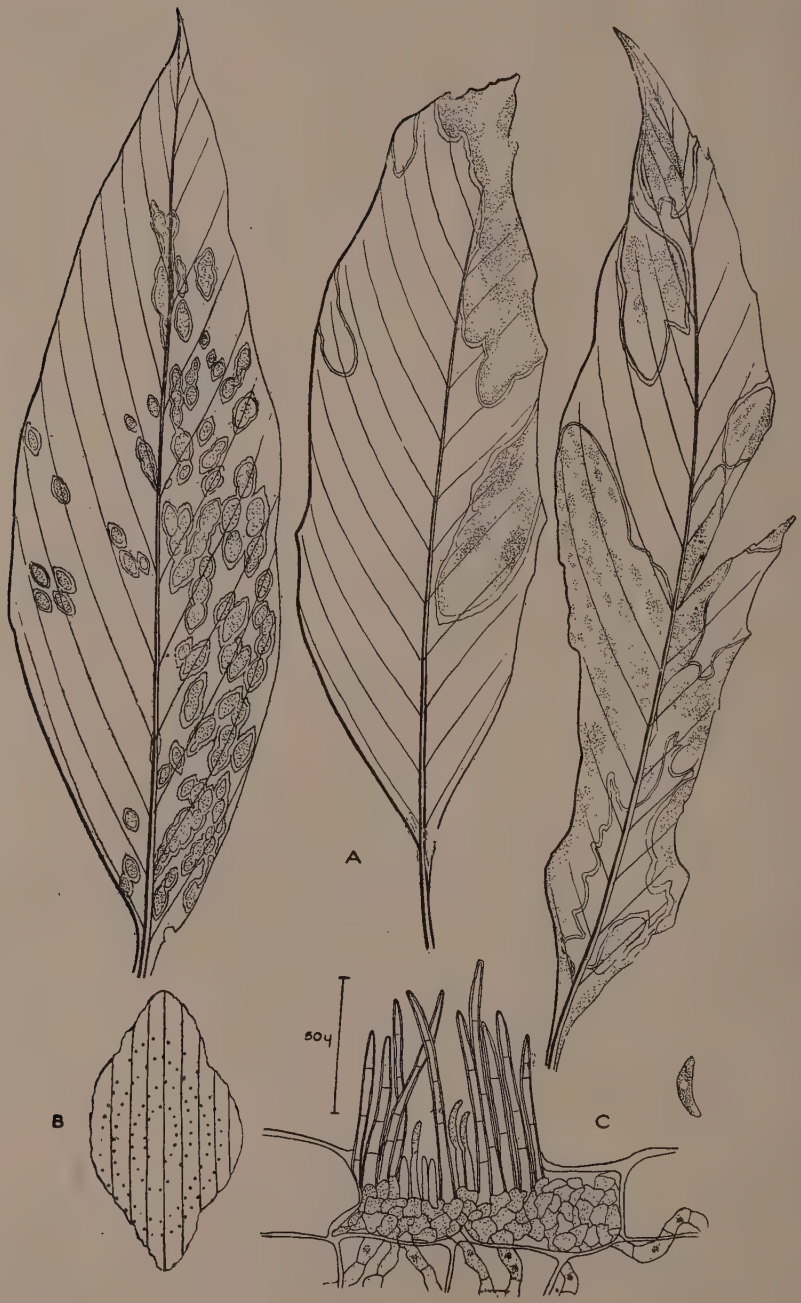
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References

Butler, E. J. & Bisby, G.R. (1931) The Fungi of India. Sci. Monogr. 1: I.C.A.R. p. 152-153.

Ramakrishnan, T.S. (1941) Studies in the genus *Colletotrichum* I, Proc. Indian Acad. Sci. B. 13: 60-70.

——— ——— (1947) Studies in the genus *Colletotrichum* III, Ibid. 25 : 15-27.

Sampson, H.C. (1936) Cultivated crops of British Empire. Bull. Misc. Information, Kew. p. 62.

Sundararaman, S. (1925) *Vermicularia curcumae* Syd. on *Curcuma longa*. Year Book Madras Agric. Dept. p. 18-19

——— ——— (1925) Rep. Sub. Officers Dept. Agric. Madras for 1924-1925, Government Mycologist. p. 290.

——— ——— (1926) Some Vermicularias of economic importance in South India. Year Book Madras Agric. Dept. p. 10-12.

Illustrations

(*a*) Turmeric leaves with spots or patches.
(*b*) One spot enlarged showing the arrangement of acervulus.
(*c*) Section of an acervulus and one conidium.

A DISEASE OF *LAGERSTROEMIA SPECIOSA* (L.) PERS. CAUSED BY *MARASMIUS CAMPANELLA* HOLTERM.

SACHINDRANATH BANERJEE AND NIRMALENDU MUKHERJEE

(Accepted for publication, October 21, 1954)

I. INTRODUCTION

In reviewing the literature pertaining to the fungus *Marasmius campanella* Holterm., it has been found that although there is a tendency towards its symbiotic mode of life on *Lagerstroemia speciosa* (L.) Pers. (=*L. flos-reginae* Retz.) as suggested recently by Bose and Chatterjee (1950), it occurs purely as a saprophyte but without any reference to the nature of decay caused by it. It was first described by Holterman (1898) from Java, as a saprophyte on dead branches. It has not yet been fully described from India and so far nothing has been published in detail on its symbiotic relationship with *L. speciosa* by Bose and Chatterjee. Field observations, however, show that its association with *L. speciosa* (Pl. I, Figs. 2–4) in localised areas of Calcutta and suburbs, is by no means universal. Like so many minor fungi its sporadic association on apparently healthy *L. speciosa* has, so far, not attracted the attention of Plant Pathologists sufficiently for them to study the problem in detail. Since the host wood belongs to one of the important timber-yielding plants in India, it is felt that any information about the fungus and its association with the host is desirable. With this object in view this investigation was undertaken in the month of July, 1952 and the results of studies done so far are summarised as follows.

II. SYMPTOMS

The outward visible symptoms of the disease are few but conspicuous. The trunks of the infected trees are badly shaped and show longitudinal crackings of the bark (Pl. I., fig. 4) which often coalesce and extend upwards to a considerable distance. They are more numerous on the main trunk, particularly towards the base than those on smaller branches. Numerous small, white fructifications of *Marasmius campanella* annually burst open through these cracked areas in the bark during the rainy season (Pl. I., figs. 2–4), but these disappear usually after the rains. On a bright sunny day, the fructifications dry up, become inconspicuous and almost always escape notice, but just after a shower of rain they quickly revive and regain their normal appearance when they can be easily detected. The underlying tissues of the bark in an advanced stage of decay die and become desiccated. The bark ultimately becomes loose, and separates easily (Pl. II, fig. 8). Patches of white mycelium are often found on the surface of the decaying sapwood and between the discoloured tissues and the bark. The sapwood in contact with the bark, shows whitish discolouration, often showing longitudinal depressions and

covered with a thin coating of whitish mycelium (Pl. II, fig. 8). The inner surface of the bark in contact with the wood occasionally exhibits white mottled appearance. The terminal portions of the branches, the young twigs, roots and young saplings, however, neither show such symptoms nor do they bear any fructification of the fungus. In the early stages of the disease, the crowns of the infected trees look apparently healthy and do not give any evidence of the disease from a distance. But it has been found that in those trees where the diseased condition has advanced to a considerable extent, there occur, frequently among the healthy branches some diseased shoots showing typical "die-back" symptoms (Pl. I, fig. 1). These branches gradually die and dry up from the tip downwards, their leaves fall off and they project conspicuously above the crown. The first sign of 'die-back' is the palloring of the leaves from the tip downwards and these gradually turn yellow and finally fall off. In standing trees the bark is considerably crinkled in an advanced stage of the disease and cracked all over its surface. In the older regions, however, the bark is severely damaged at places, thus exposing the underlying tissues which become partially desiccated. Finally, these branches dry up and can be broken off easily.

III. Materials for Study

The materials for study were collected in July, 1952, in the form of freshly cut infected branches and twigs of *L. speciosa* from the Lake area of South Calcutta with numerous white fructifications of *Marasmius campanella* thereon (Pls. I & III, figs. 3 & 17). The situation was somewhat shady and damp and, as such, during the rains, formed a favourable condition for the production of fructifications of the fungus. During this period the temperature varied between 26°—30°C and the atmospheric humidity was about 80—98%. Trees to be studied in this connection were carefully selected and infected branches of various ages were cut down in order to examine macroscopically the nature of decay in the wood. Small, cylindrical sections from the selected portions of the same or different branches, were brought into the laboratory, examined and studied while in a fresh condition. The cross-section ends of these samples, when cleaned and plained, showed the presence of decay at various stages in the wood. Microscopic examinations of the rotted areas showed abundant hyaline mycelium with clamp-connections within the tissues of the host.

IV. Decay of *Logerstroemia speciosa*

A. Macroscopic Characters of the Rot: Cross-sections of the wood in the early stages of decay show the presence of somewhat yellowish or pale brownish irregular areas at the periphery of the sapwood, each being bound internally by a narrow brownish invasion zone. The wood when longitudinally cut shows incipient decay in its early stage by the presence of bleached elements extending vertically through the sapwood (Pl. I, Fig. 7). The rots gradually extend horizontally towards the centre of the wood, enlarge and other light coloured areas also appear, which later coalesce to form

a continuous area of discoloured but firm sapwood (Pl. I, figs. 5 & 6). In such cases it is obvious that the course of infection is from the bark to the centre of the heartwood. With the gradual advance of decay there appear at the periphery of the sapwood minute rot-pockets filled with either whitish mycelium or decayed materials (Pl. I, fig. 6). With age these increase in number, unite and form larger pockets. The rot seems to spread much more rapidly in a longitudinal direction than laterally. With age there is appreciable decrease in strength of the wood which offers less and less resistance to cutting and breaking. The sapwood particularly becomes very light, soft, spongy and brittle. In an advanced stage the rots show various stages of decay from the periphery towards the centre of the heartwood in which there appear whitish or pale yellowish irregular areas with small pockets which may or may not contain white material while in others these are reduced to a mess of whitish or pale coloured fibres. Finally, the wood crumbles easily and thin sheets of conspicuous whitish mycelium are found along the grains of the wood.

B. Microscopic Characters of the Rot: In search for the mycelium in the diseased wood for the purpose of studying its nature and distribution, infected wood in all stages of decay were sectioned both free hand and with the help of a microtome. In most cases radial, tangential and transverse sections of sound and diseased wood, about 15-20 μ thick, were cut without any special treatment, from freshly cut branches with a view to avoid drying out of the wood and consequent shrivelling up of the fungal hyphae. When it was found necessary to cut sections from partially dried wood, wood blocks were given some preliminary softening treatment by keeping them for 2-3 days in equal parts of methylated spirit and glycerine, or putting the blocks in boiling water until they sank. In such cases the material was not allowed to stand long enough in water but sections were cut immediately and their surfaces had been kept wet with water during the process. Sections of wood from freshly cut branches were however found to be in a better condition and the mycelium in the wood elements was quite suitable for study. In order to study hyphae in detail within the elements of the wood, differential staining methods by Gentian violet and Bismarck brown as recommended by Hubert (1922) and Safranin & Picro-aniline-blue recommended by Cartwright (1929) were tried. Of the two, the latter method was found to be more satisfactory and easy to manipulate. By this method the lignified structures were stained red and the mycelium blue.

The distribution of the mycelium in the decayed wood is fairly uniform. In the early stages of the decay, the hyphae are often scanty but later they become plentiful and distributed throughout the elements of the wood. At first the hyphae develop mainly in the medullary rays and to a certain extent within the vessels (Pl. II, fig. 9), but in the wood-fibres they are comparatively less numerous. The hyphae often fill the entire cavities of the elements forming wefts and are of two kinds; firstly, rather fine, profusely branched, hyaline, with a few clamp-connections, about 1-2 μ across and secondly, a

coarser hyaline hyphae, sparingly branched, with frequent. simple clamp-connections, with conspicuous granular contents and about 2.5-3.5 μ across.

Double clamp-connections, which are so common in artificial cultures, have not, however, been observed. The finer hyphae appear to be more numerous in the medullary-ray-cells and wood-fibres and probably are the first to invade the uninfected parts. These can be demonstrated readily by differential staining of sections of wood in the incipient stage of decay where coarser hyphae are entirely absent. That these hyphae belong to *Marasmius campanella* was proved by isolating the same in culture. The hypae at first ramify and pass from cell to cell mainly through the simple pits of the wood elements (Pl. II, fig. 9) but later they directly penetrate the cell-walls forming numerous bore-holes (Pl. II, fig. 9). In passing through the cell-walls, the finer hyphae show little or no diminution in their width, but the apex of the wider hyphae at first becomes very much attenuated like a peg during the process of penetration. The bore-holes are formed in advance by enzymic action at the point of contact of the hyphae and during the process ot penetration it is found that they are clearly visible within the wider bore-holes through which they passively pass. At first these bore-holes though wider than the penetrating hyphae are merely fine passages which at length enlarge in diameter. In case of wider hyphae its attenuated apex, after penetration of the wall, begins to widen till it attains its normal diameter, while the portion of the hyphae remaining within the bore-hole appears exceedingly fine. Bore-holes in advance stage of decay become more numerous and often coalesce to form irregular holes. At first they appear round or oval in form and are about 1-2 μ across. Their inner contours are smooth and not irregularly ruptured. This fact clearly indicates that they have originated due to the dissolution by a chemical solvent reacting from a central point. This type of chemical dissolution of the cell-wall by enzymic activity of the fungus supports Proctor's (1941) theory of cell-wall penetration.

C. Microchemical Studies: Microchemical tests for the detection of lignin and cellulose present in the sound and partially decayed wood have been made. This has been done in the usual way by staining the sections of wood with Phloroglucin-HCl for lignin and Chlor-zinc-iodine for cellulose. The combination stain, Gentian violet and Bismarck brown which differentiates lignified (violet staining) and non-lignified (brown staining) structures has also been tried. Other microchemical stains have also been employed most of which agree closely with the changes indicated by the use of above-mentioned stains and will, therefore, not be referred again. However, in every case, if possible, a chemical analysis of the decayed wood is desirable in order to corroborate the changes indicated by these stains. Nevertheless, the above mentioned stains are still in vogue in such work to indicate the presence of lignin and cellulose. The results (obtained so far) are summarised below.

Results of staining with Gentian violet and Bismarck brown for cellulose and lignin in normal and partially decayed wood:—In the

normal wood, the secondary walls of all the wood-elements are variously stained from brown to yellow through the intermediate shades of brownish-yellow and yellowish-brown suggesting the presence of cellulosic materials in them. The middle-lamellae of all the tissue-elements, take up deep violet to violet stains. The secondary walls are, however, partly light violet in the fibres, tracheids, vessels and wood-parenchyma and light-violet in most of the ray-cells. These suggest that lignin is present to a considerable extent in the middle lamellae and to a less degree in the secondary walls of all the elements. In the partially decayed wood, the brownish yellow colour is more noticeable along with different shades of violet colouration throughout the section. The middle-lamellae also retain their violet colour. No remarkable change in the colour of the wood-parenchyma is observed. Partly digested inner walls of the fibres appear somewhat yellowish-brown. The ray-parenchyma remains almost in the same state as in the sound wood.

Results of staining for cellulose and lignin in normal and diseased wood with Chlor-zinc-iodine and Phloroglucin-HCl respectively :—In normal wood with Phloroglucin-HCl, the middle-lamellae of all the cells are stained deep red. In the vessels, wood-parenchyma and ray-cells, the secondary walls are generally stained red. In the fibres, however, the secondary walls become faint pink. With Chlor-zinc-iodine only, the secondary walls of the fibres take up blue colouration and those of the ray-cells and wood-parenchyma partly blue. In the partially decayed wood, all the cells show red colouration in the primary walls with Phloroglucin-HCl. The secondary walls of the vessels, wood-parenchyma and ray-cells also show red colouration. In the fibres and tracheids the secondary walls are found to undergo dissolution to some extent, the fragments remaining, however, may either take up a faint pink colour or become almost colourless. This is the only change that can be recognised in the secondary walls of the tracheids and fibres. The result obtained with Chlor-zinc-iodine, on the other hand, also points to the fact that the apparent change from the normal wood has taken place also in the fibres and tracheids. The secondary walls of both these elements have taken up blue colouration. The pale blue and pink colouration shown by the walls of the tracheids and fibres with Chlor-zinc-iodine and Phloroglucin-HCl respectively, however, point to the fact that in the normal wood these elements are slightly lignified. In the diseased wood many of the fibre-walls do not show any pink colouration, but take up deep blue colour, suggesting absence of lignin. Thus, it can be concluded that in the decayed wood there is considerable delignification of the lightly lignified walls of the fibres and tracheids together with the loss of cellulosic materials to some extent from the wood-parenchyma, ray-cells, etc.

Sulphuric Acid Test :—Sections of normal and partly decayed wood corresponding to those used for microchemical stains have been placed on slides and treated with 72% H_2SO_4 as recommended by Ritter (1925). In the section of decayed wood the fragments of the secondary walls of the fibres and tracheids have been dissolved and disappeared and slight swelling and distortion of tissues have been

observed. A net-work of middle-lamellae, isolated vessels, ray-cells and wood-parenchyma are left in place on the slide. The sections of normal wood, on the other hand, when treated in the same manner react violently. The cells swell rapidly, break apart, the secondary walls of the fibres and tracheids dissolve and a mixture of floating lamellae, vessels, ray and wood-parenchyma cells are left on the slide. In this case much distortion of tissues takes place due to violent action of sulphuric acid on cellulose indicating the presence of large amount of cellulosic materials in the cell-walls of the normal wood. The swelling of this cellulose preceeding dissolution due to action of sulphuric acid forces all the tissue-elements apart. In the partially decayed wood the action is mild and the tissue-elements are in a slightly distorted position, indicating the presence of less cellulosic materials and hence there is less violent chemical reactions. The test in itself is not, however, a conclusive one, but it undoubtedly indicates the presence of less cellulosic materials in the partially decayed wood caused by *Marasmius campanella*, than those in the normal wood.

V. The Causal Agent

A. Isolation and Identification of the Causal Organism

The mycelium from the tissues of the host was isolated in the usual way and studied. These transfers were kept under ordinary conditions of temperature (29°—30°C) humidity (80—90%) and diffused light of the laboratory. In most cases mycelia grew out into the medium within a week and from these several sub-cultures were made which were used subsequently as stock cultures. After close examinatons and further study of sub-cultures of all the isolates it was found that majority of these cultures belonged to one and the same fungus and these later exhibited all the cultural characteristics of *Marasmius campanella*. Attempts were, however, also made to isolate the fungus from different parts of healthy seedlings and seeds of *L. speciosa* obtained from the Forest Department, Government of West Bengal, but without any success.

The isolates of *Marasmius campanella*, were subsequently compared with the polysporous cultures of the same fungus already made available for the purpose and found to be identical with the latter in both cultural and microscopic details. The identity of these isolates was further confirmed when they produced typical fructifications in artificial culture (Pl. II, fig. 10).

B. The Sporophore.

PILEUS.—Stipitate, usually with an excentric to rarely central stipe, occasionally laterally stipitate, always centrally stipitate in culture ; at first hemispherical, then more or less expanded and convex, at maturity becoming flat to somewhat concave with reflexed margin ; coriaceous when dry, reviving with moisture and becoming somewhat sub-gela-tinous ; about 5 to 16 (20) mm. in diameter ; margin thin, at first involute, becoming straight to reflexed, when fully expanded entire, extending beyond the gills, white in colour.

Upper Surface.—Smooth, soft, somewhat water-soaked in appearance; at first pinkish in some cases, frosty-white at maturity, becoming buff-coloured on drying, sometimes yellowish at the centre with a slight depression, outer portions remaining white.

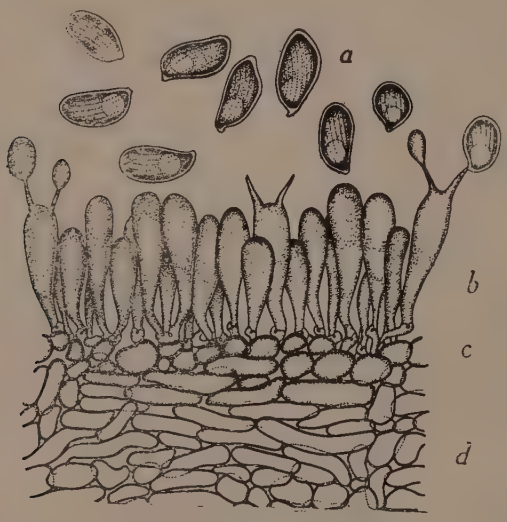
Flesh.—Thin and membranous; less than a mm. thick; subgelatinous, coriaceous on drying; frosty-white in colour.

Gills.—Adnate, broadly attached to the stipe; broadest in the middle but gradually narrowed towards the margin; about 5-15 mm. broad; number of gills varying from 8-18 per pileus, of two lengths, longer gills alternating with the shorter ones; at first pinkish, then frosty-white; becoming somewhat yellowish with age.

Trama.—Consisting of loosely interwoven hyphae; cells mostly without appreciable granular contents, walls partially gelatinised; about 20-57-73 μ wide (Text-fig. 1, d).

Sub-hymenium.—Consisting of one or two layers of almost rounded hyphal cells (Text-fig. 1, c).

Hymenium.—Consisting of mature and immature basidia of different generations; arising singly or in groups from the apex of a hypha and each with a clamp-connection at the base; about 18-22 μ wide (Text-fig. 1, b).



TEXT FIG. 1.

Basidia.—Clavate; disterigmatic and bisporous; dimension about 18-22 x 4-5 μ at maturity; sterigmata two, about 6.5-7 μ long (Text-fig. 1, b).

Basidiospores.—Hyaline; slightly thick-walled; wall faintly striate; usually oval or oblong, rarely narrowed at both ends, sometimes rounded at the apex and gradually narrowed towards the base, often flattened at one side, with a distinct apiculus; full of granular contents at the upper end and usually with a large vacuole towards the base; ordinarily binucleate, rarely uninucleate; dimension about 2.5-4.5 x 2-3.5 μ (Text-fig. 1, a).

Cystidia.—Not found.

STIPE.—Usually excentric, rarely central, occasionally lateral; more or less cylindrical with a somewhat swollen base; coriaceous; firm; solid; not easily separable from the substance of the pileus; without annulus or volva; surface smooth and white throughout excepting the finely hairy swollen base; becoming buff-coloured on drying; dimension about 3-8 (20) x 1-1.5 (2) mm.

Two types of hyphae have been found to enter into the composition of the fructification, *viz.*, (i) thin-walled, hyaline, wider hyphae, straight, branched and septate with dense or sparse granular contents, sometimes empty, with frequent, simple or double clamp-connections, walls often partially gelatinised, about 2.5-11.5 μ wide, and (ii) comparatively thick-walled, hyaline, narrow to wide hyphae, straight or slightly flexuous, sparingly branched, with frequent simple or double clamp-connections, with a few granular contents, sometimes empty, wall-thickness variable, lumen either wide or almost obliterated and, about 4-14.5 μ across. The transitional stages between these two types of hyphae can be easily recognised. The two kinds of hyphae are characteristic of the stipe and the pileus. In different parts of the pileus the wider hyphae are abundant and the wider empty hyphae with partly gelatinised walls are more numerous than those in the stipe (Pl. 4. Fig. 18, r-s). Near the hymenium the basidia bearing hyphae show abundant simple, double or sometimes whorled clamp-connections, so that the basidia often appear to grow in clusters of two or more at the apex of a hypha. In the stipe the same type of hyphal organisation can be observed but the two types of hyphae run more or less parallel to one another and grow vertically upwards although the partially gelatinised hyphae are not uncommon (Pl. 4. fig. 18, p-q). In the 'button-stage' of the fruit-body the same type of hyphal organisation has been observed but both types of hyphae are narrower, more flexuous, and the presence of empty hyphae with partially gelatinised walls are not much in evidence (Pl. 4, fig. 18, n-o).

C. Fungus in Culture

(a) *Spore-culture*

The cultures of *Marsamius campanella* were not only made from the rotted tissues of the wood but also from the spores. Initial polysporous cultures were made by transferring the spores aseptically

to 2.5% malt agar slants and incubated at 30°C in complete darkness. Within a week, a good mycelial growth was obtained in all the tubes and these were kept as stock-cultures for study and comparison. For making comparative cultural study several monosporous cultures of the fungus were also made. A spore suspension in sterile distilled water was spread on cleared agar plate and the superflous water was carefully drained off. After about 6-8 hours the plates were examined and well-germinated spores were located under the microscope. These were then isolated by punching out small discs of agar each containing single spore by means of a sterile dummy objective and transferred to 2.5% malt agar slants.

(b) *Spore-germination*

The spores used for the following experiments were obtained both from the sporophores collected in the field and from those obtained in pure cultures grown on Badcock's medium (1943) and potato-dextrose agar. Spores were sown in hanging drops of distilled water (pH 7) and tap water (pH 6.8) in the usual way in sterile van Teieghem cells. Germination of spores was also tried on 2% malt agar (pH 6.7) and potato-dextrose agar (pH 6.7) in sterile Petri-dishes or by following Kniep's agar-film technique (1913) as modified by Banerjee (1951). In such cases thin spore-deposits were directly taken on the media from the sporophores fixed to the inner side of the upper lids of the Petri-dishes. All these were kept inside bell-jars during observation under ordinary conditions of light (diffused), temperature (28°—29°C) and humidity (83-85%) of the laboratory.

In all cases germination starts within 1-2 hours after planting the spores in water or upon the agar media. The percentage of germination is very high. In general, germinating spores swell considerably and the germ-tubes appear usually at one end of each spore, sometimes from its side. Following the first germ-tube, a second germ-tube, sometimes appear at the base of the same germ-tube simultaneously, of which one soon takes the lead. The germ-tube develops directly into the secondary mycelium characterised by the comparatively wide hyphae with cross-walls and with frequent clamp-connections. In rare cases, however, the spore on germination also gives rise to primary mycelium without clamp-connections and with comparatively few cross-walls. Details of nuclear phenomena of spore-germination and in the primary and secondary mycelia have also been studied and the observations will be communicated in details elsewhere.

During these experiments it has been observed that the spores can even germinate well on perfectly dry slides within 24 hours after spore-fall under identical conditions of light and temperature provided the humidity of the surrounding atmosphere is near about 90% or more. In controlled humidity, it has been noticed that the germination of the spores on dry slides gradually decreases as the percentage of relative humidity comes down. At 60% relative humidity the germination is altogether stopped.

(c) *Oxidase Test*

It is well-known that the intensity of the attack on lignin is directly dependant upon the oxidase activity of the white-rot fungi. Bavendamm (1928) described a test by which dark rings appear round the inocula due to the presence of oxidases, secreted by the white-rod fungi and these can be detected by growing the fungi on malt agar medium containing 0.5% gallic or tannic acid. This test has been performed with *Maramius campanella*, and it has been found, that it being a white-rot fungus can produce such rings and therefore, is capable of decomposing lignin. In both cases, chocolate-brown rings appear round the inocula within 24 hours after inoculation but their intensity of reaction shows some variation. On tannic acid medium the reaction is intense to very intense and the diameter of the ring is 15-17 mm. On gallic acid on the other hand, the reaction is mild and the diameter of the ring varies between 11-15 mm.

(d) *Cultural characteristics.*

So far as the authors are aware, nothing is known about the cultural characteristics of *Marasmius campanella.* For studying its cultural characteristics, the mycelium isolated from the decayed wood as well as the polysporous cultures were grown on potato-dextrose agar and malt agar (2.5% malt, agar 2% and distilled water 100 c.c.) media. Potato-dextrose agar was prepared according to the recipe of Fritz (1923). They were grown in Petri-dishes and kept in darkness, as well as, in diffused light of the laboratory at a constant temperature of 30°C. The cultures received diffused light for about six hours everyday during the experimental period. The pH value of each medium was adjusted and after sterlization was found to be 6.2. The terms used to describe the texture of the mat are those proposed by Long and Harsch (1918) and Nobles (1948). Since the cultural characteristic of both the isolates and the polysoporous mycelium have been found to be identical, no attempt has been made to describe their characters separately.

(i) *Habit of growth* (Pl. II, figs. 11 & 12).—*On potato-dextrose agar* growth starts with much rapidity in all cases forming a white, raised, cottony mycelium over the inoculum and spreading in all directions without any appressed advancing zone within 24—28 hours after inoculation. As the growth advances, the texture of the cottony mycelium becomes somewhat silky-wooly in appearance. Although in light the growth appeared to be more rapid than that in darkness in early stages, but later, the growth becomes more uniform under both the conditions. In about five days after inoculation, the mat proper condenses and differentiates into a broad central white, felty zone around the inoculum and a broad woolly peripheral region. At this stage the surface of the medium is completely covered and the growth is so vigorous that the mycelium tends to cover the bare inner glass surface of the upper lid of the Petri-dish. Condensation of the mat continues and its peripheral portion assumes a woolly-felty texture in about nine days after inoculation. Finally, the texture of the mat becomes felty with age. *On malt agar* no appreciable

difference in growth characters is noticed but the mycelium appears to be more compact and tends to remain more or less flat from the early stages.

(ii) *Colour.* On both the media all the cultures remain white throughout and no other pigment develops even in cultures 45-days-old. Later, in about 2-3 months after the inoculation, the whole mat turns somewhat light buff in colour. At this stage the colour of the medium is also changed.

(iii) *Rate of Growth.* —The rate of growth on both the media is rather slow to start with but increases gradually and the daily increment in diameter of the aerial mycelium in Petri-dish cultures has been determined. The radial growth in all cases in light and darkness are identical and average measurements of several cultures have been taken. The difference in growth on potato-dextrose agar and malt agar is shown in the following table :—

TABLE I. *Average daily increment (mm.) of growth*

Media	No. of days and growth rate.				
	1	2	3	4	5
Potato-dextrose agar.	5.5	11.5	16.5	17.5	Covered.
Malt agar.	4	9.5	11	11	11

From the table it appears that the fungus grows more rapidly on potato-dextrose agar (Pl. II, fig. 12) than on malt agar (Pl. II, fig. 11).

(iv) *Hyphal characters* (Pl. IV, fig. 18, a-m)

Advancing zone. Two types of hyphae can be recognised *viz.*, (i) much branched, hyaline, thin-walled hyphae, usually flexuous, sometimes more or less straight, with frequent simple or double clamp-connections and full of granular contents, about 2.5-3μ across; and (ii) a few thin-walled, hyline, wider hyphae, more or less straight to slightly flexuous, distantly branched, branches arising at acute angles with the parent hypha, full of granular contents, with infrequent simple or double clamp-connections, and about 6-6.5μ in diameter. Intergrades between the two types were clearly evident, the second type often giving rise to the first (Pl. IV, fig. 18 a-d).

Aerial mycelium. Same two types of hyphae present; wider hyphae becoming more frequent and branching more close; a few clamp-connections present; full of granular contents and about 2.5—7μ in diameter with the usual intergrades (Pl. IV, fig. 18 e-i).

Submerged mycelium. Narrower hyphae comparatively few, about 2-3μ across; wider hyphae predominating, much branched and

flexuous, clamp-connection rare, about 5-6μ in diameter; intergrading hyphae common, in most cases full of granular contents, a few wider hyphae showing a tendency to become almost empty. (Pl. IV, fig. 18 j-m).

VI. Inoculation Experiments

Inoculation experiments with *Marasmius campanella* were carried out in the laboratory as well as in the field. In the laboratory, following the method suggested by Brooks & Moore (1923), freshly cut young twigs of *L. speciosa*, about 4" long, were inoculated. One end of the cut twigs was kept immersed in water in a beaker. Their upper ends were cut off and spore-suspensions of *Marasmius campanella* in sterile distilled water were applied to the freshly exposed surface and never allowed to dry out. The beaker was then placed inside a bell-jar under ordinary conditions of diffused light and temperature (28°—29°C) of the laboratory. Since the spore of *Marasmius campanella* germinate within a short time, longitudinal sections were cut through the inoculated ends of the twigs at intervals of 6, 12, 24, 48, 72 and 96 hours after inoculation. Sections cut after 6 and 12 hours showed that the majority of the spores had germinated on the cut surface and had sent hyphae down into the elements of the wood ultimately forming a vigorous mycelium in about 96 hours. Some of the spores had also been sucked inside the vessels and germinated there. The cut twigs, however, could not be kept in an actively growing condition during the experimental period. As such, their invasion by the germinating spore of *Marasmius campanella* could not be regarded as an evidence for its parasitic action on the trees. Nevertheless, it could be assumed that if by chance the spores some how alight on the surface of the wound of a living tree in the field, they could germinate on the surface under suitable conditions producing infection threads which in some way or other penetrate the elements of the wood.

Field inoculations were started on healthy living trees of *L. speciosa* at the Indian Botanic Garden, Sibpore, Howrah, in January, 1953. Six inoculations were made on some of the stout branches, about 1½—4" in diameter, keeping adequate controls. The procedure involved was to make an incision resembling two upper arms of a triangle through the bark (Pl. III, fig. 13) and the branches were inoculated by transferring bits of actively growing polysporous mycelium from culture tubes into the wound by carefully lifting the flap of the bark still attached to it. Prior to inoculation, the surface of the branch was wipped out with rectified spirit and subsequently washed with sterile distilled water in order to free the surface, as far as possible, from surface contaminations. Each wound was then covered with moist absorbent, sterile cotton-wool with a piece of thick paper over it in order to prevent drying out and firmly bound with a string. The string and the cotton-wool were removed after a month and the inoculated branches were left exposed in that condition. At this stage no external sign of infection was, however visible.

After a period of eight months, the branches were found to take up the infection. The infected wounds became very conspicuous and

there was considerable amount of swelling of the wood which protruded through the wounds (Pl. III, fig. 16). Macroscopic examination of the sections of the branches at the point of infection showed the characterstic rot in the sapwood in its early stages (Pl. III, fig. 14-15). The invaded wood was discoloured and there was considerable paling of the bark around the wound. The controls, however, remained flat and there was no discolouration of the wood below the bark. In one case, one fructification of *Marasmius campanella* appeared over the swollen portion of the wound. No other external indication of decay was, however, noticed. As such, the internal conditions of the infected branches were examined. The mycelium had spread approximaely about 1.5″ above and below the point of infection. Spread in the transverse direction was about ½″ and that in the lateral direction about 1.5″. Mycelium was found within the tissues of the branches inoculated although its distribution within the tissues of the host was not uniform. The hyphae were abundantly present below the bark and in the living cortex and the peripheral portion of the wood. The inner sapwood was without any trace of hyphae. Very little can be said at present as to the ultimate effects produced by the advanced mycelium and the greatest distance to which the mycelium had penetrated into the wood from the point of infection. However, all these point to the fact that *Marasmius campanella*, under favourable conditions is able to infect living trees of *L. speciosa*. This was further confirmed by re-isolating the fungus from different newly infected lesions. Further, it can also be stated from the progress it has made within a limited period of eight months' study that the fungus is not a strong parasite owing to its slow growing nature within the tissues of the host.

VII. Discussion

A study on the biology of *Marasmius campanella* Holterm. and its sporadic occurrance on the living trees of *Lagerstroemia speciosa* induces the writers to discuss some aspects on the relationship of the fungus with the host. During the rainy season fructifications of the fungus are found to crop up on the trunk and also on older, younger and dead branches of apparently healthy trees so that the fungus may be considered as a parasite. As to the parasitism of a wood-rotting fungus, at present, there exists two schools of thought. Some consider that parasites are those which grow only on the living trees. Others are of opinion that parasites, in the strictest sense of the term, should derive their nourishment from the living cells of the host. So, before calling a fungus a parasite, it is essential to detect the presence of living tissues in the infected regions of the host. In case of *L. speciosa*, on microscopic examination, the living elements have been found to be the wood-parenchyma and ray-cells in the infected outer sapwood. It is not, however, justified to call a fungus a parasite from its mere presence in the sapwood, as it may not necessarily invade the parenchyma and ray-cells, the ultimate death of which may be due to the disintegration of the already infected dead elements lying in between them within the sapwood.

From the inoculation experiments carried out in the field on healthy branches of *L. speciosa* it can be said that the cambium being killed at the place of infection, the fungus can establish itself in the living tissues of the sapwood through the wounds in the bark. Both intercellular and intracellular mycelia have been noticed. Here the infection proceeds from the bark to the outer woody portion of the stem. But this may not be the case in nature where the point of infection may not be of the same nature. It may be on the bark or directly on freshly exposed woody tissues of the host. Irrespective of the place of infection, it has been found that mycelium grows more in the longitudinal direction than horizontally or laterally, although the rate of growth is very slow. It has been experimentally shown that, under controlled conditions freshly cut ends of the twigs of *L. speciosa* can suck spores of *Marasmius campanella* which germinate inside the woody-elements. The spores can also germinate on the cut-ends of the twigs and send down hyphae into the tissues of the host. So, it is evident that in such cases the woody elements are the first to be attacked from where the mycelium may or may not proceed towards the periphery. The steps towards the advancement of infection in such cases in the field could not, however, be traced. From the results of inoculation experiments so far made, it can be said that *Marasmius campanella* can be regarded as a wound parasite, killing the living cells of the normal vigorous trees though it is not virulent in its parastic activity.

Whenever the fructifications of the fungus were collected, they were found to grow in profusion in the conspicuous furrows of the highly cracked bark on the main trunk or on the young apparently healthy branches. The fructifications were also found on the bark which did not possess visible cracks that are common on the main trunk or older branches. In such cases no superficial mycelium either on the bark or in contact with the sapwood underlying the bark was, however noticed. But microscopic examinations of the peripheral tissues underlying the bark in such areas show plenty of fungal hyphae with clamp-connections. Mycelial sheets were only found in the highly advanced stage of decay when the underlying tissues of the bark had completely distintegrated and the bark separated easily.

L. speciosa is chiefly found on the road-sides of Calcutta. It has been observed that the plants which grow in comparatively damp and shady situations are more attacked by the fungus than those which grow in dry and uncongested areas. It may be suggested that the infection of healthy trees possibly takes place in the following way. In the rainy season, in a stormy weather usually due to the high velocity of the wind the branches of *L. speciosa* may crack or break down forming fresh wounds. The spores from the fructifications of infected trees situated nearby are blown away by the wind and taken over to the newly exposed wounds of healthy trees, where they alight, germinate and make new infections even in the absence of water provided the surrounding humidity of the atmosphere is very high.

VIII. Summary

1. As nothing so far has been done as to the exact relationship existing between the fungus *Marasmius campanella* and *Lagerstroemia speciosa*, this investigation had been undertaken. Although, so long recorded as a saprophyte, it has now been proved to be a week parasite on its host.

2. The external and internal symptoms of the disease have been fully described. Field observations have shown that the fungus preferably lives in the sapwood but it can also penetrate the heart-wood.

3. Chemical changes in the wood during decay has also been noted by the usual microchemical staining methods. In partially decayed wood the change consists in the gradual delignification of the lignified secondary walls of the wood-elements. The cellulosic materials mostly remain and show better reactions than those in the normal wood.

4. The external morphology of the fructifications of the fungus obtained from nature as well as from cultures has been fully described and their anatomical pecularities noted. The basidia have been found to be disterigmatic and bisporous,

5. Fructifications obtained for this investigation were found to grow on the main trunk bursting open through the decayed or cracked bark or on the bark of comparatively younger and stout branches. On close examination the younger branches were generally found to be associated with injuries on bark which may be regarded as the places through which infection of healthy trees by the fungus has possibly taken place.

6. Basidiospores have been found to germinate very quickly in water and on agar media under ordinary conditions of light and temperature of the laboratory.

7. Cultural characteristics of the fungus have been studied on two different media. Mycelia have been found to grow with greater rapidity in potato-dextrose agar than those on malt agar. Being a white-rot fungus it gives positive reaction to Bavendamm's oxidase test.

8. Inoculation experiments on healthy branches of standing *L. speciosa* in the field has shown that the fungus can establish itself in the living tissues of the host, through wounds in the bark. However, the advancement of the mycelium within the host-tissues has been found to be very slow. Experimental results have also shown that the spores of *Marasmius campanella* can germinate well on the freshly cut-ends of the twigs of *L. speciosa* and infection threads penetrate the tissues of the host eventually forming profuse mycelium within the wood-elements. Some of the spores also pass down the wood-elements and germinate there. From these experimental results it can be said that *Marasmius campanella* is a wound parasite on normal vigorous trees.

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REFERENCES

Badcock, E. C. (1943) Methods for obtaining Fructifications of Wood-rotting Fungi in culture. *Trans. Brit. Mycol. Soc,.* **26**, 127-132.

Bavendamm, W. (1928) Neue Untersuch ungen uber die Lebensbedingungen holzzersto render. *Pilze. Zbl. Bakt.*, **11**, **76**, 172-277.

Banerjee, S. N. (1951) Studies on the biology of *Auricularia auricula-Judae* (Linn.) Schroet, (= *Hirneola auricula-Judae* (Linn.) Berk) causing rot in elder (*Sambucus nigra* L.) *Part of the Ph. D. Thesis, University of Edinburgh.*

Bose, S. R., & Chatterjee, P. (1950) A case of apparent symbiosis of *Lagerstroemia flos-reginae* Retz. with *Marasmius campanella* Holt. *Proc. Ind. Sc. Cong.*, **3**, 56.

Brooks, F. T. & Moore, W. C. (1923) On the invasion of woody tissues by wound parasites, *Proc. Camb. Phil. Soc.* (*Biol. Sci.*) **1**, 56-58.

Cartwright, K.St.G. (1929) A satisfactory method of staining fungal mycelium in wood sections. *Ann. Bot., Lond.*, **43**, 412-413.

Fritz, C. W. (1923) Cultural criteria for the distinction of wood-rotting fungi. *Trans. Roy. Soc. Can. V.* **17**, 191-288.

Holterman (1898) *Mykol. Unters. ausden Tropen, 105, tab. 12.* Cited in *Sacc.*, **16**, 60.

Hubert, E. E. (1922) A staining method for hyphae of Wood-inhabiting Fungi. *Phytopathology*, **12**, 440.

Kniep, H. (1913) Birtrage zur Kenntnis der Hymenomyceten, 1, 11. *Z. Bot.*, **5**, 593-637.

Long, W. H., & Harah, R. M. (1918) Pure cultures of wood-rotting fungi on artificial media. *J. Agric. Res.*, **12**, 33-82.

Nobles, Mildred K. (1948) Studies in Forest Pathology VI. Identification of cultures of Wood-rotting Fungi, *Canad. J. Res., C*, **26**, 281-431.

Proctor, P. (1941) Penetration of the walls of wood cells by the hyphae of wood destroying fungi. *Yale Univ. Sch. For. Bull.*, **47**, 1-31.

Ritter, G. J. (1925) Distribution of lignin in wood. *Industr. Engng. Chem.*, **17**, 1-194.

Explanation of Plates

Plate I

Fig. 1. Some dead branches projecting upwards above the crown of a living *Lagerstroemia speciosa*, (=*L. flos-reginae*) showing typical 'die-back' symptom.

Fig. 2. Numerous fructifications of *Marasmius campanella* growing all over the trunk of the same living tree.

Fig. 3. Typical fructifications of *M. campanella* showing various stages of development on the trunk.

Fig. 4. Fructifications of *M. campanella* bursting open through the cracked bark of the trunk.

Figs. 5 & 6. Cross-sections of the branches of *L. speciosa* showing various stages of decay.

Fig. 7. Longitudinal section of the wood showing pale coloured rotted areas at the margin.

Plate II

Fig. 8. A short cylindrical portion of a stout branch showing the outer surface of the discoloured sapwood with mycelial mats and longitudinal depressions. ($\frac{1}{2}$ Nat. size).

Fig. 9. Radial longitudinal section through the decayed wood showing the distribution of hyphae within the vessel; numerous bore-holes and hyphae with clamp-connections can be clearly seen (X 320).

Fig. 10. Formation of a fructification of *M. campanella* from the isolates.

Fig. 11. Cultures of *M. campanella*, two-days-old, on malt agar at 30°C in diffused light.

Fig. 12. Cultures of *M. campanella*, two-days-old on potato dextrose agar at 30°C in diffused light.

PLATE III

Figs. 13 & 16. Inoculations on the branches of a healthy *Lagerstroemia speciosa* showing 8-months-old infection with *Marasmius campanella*.

Fig. 14. Cross-section of a branch of *L. speciosa* through the area of infection showing the rot in the sapwood (a).

Fig. 15. Longitudinal section through the area of infection showing the rot near the periphery extending upwards and downwards from the point of infection (a-b).

Fig. 17. Fructifications of *M. campanella* showing upper and lower surfaces (½ Nat. size).

PLATE IV

Fig. 18. Mycelial hyphae (a-m) and hyphae of the sporophore (n-s): hyphae of the advancing zone (a-d), aerial mycelium (e-i) and submerged mycelium (j-m); hyphae in the 'button-stage of the sporophore (n-c); hyphae from the stipe (p-q) and those found in the different regions of the pileus. (r-s). (X 460).

PLATE I

PLATE II

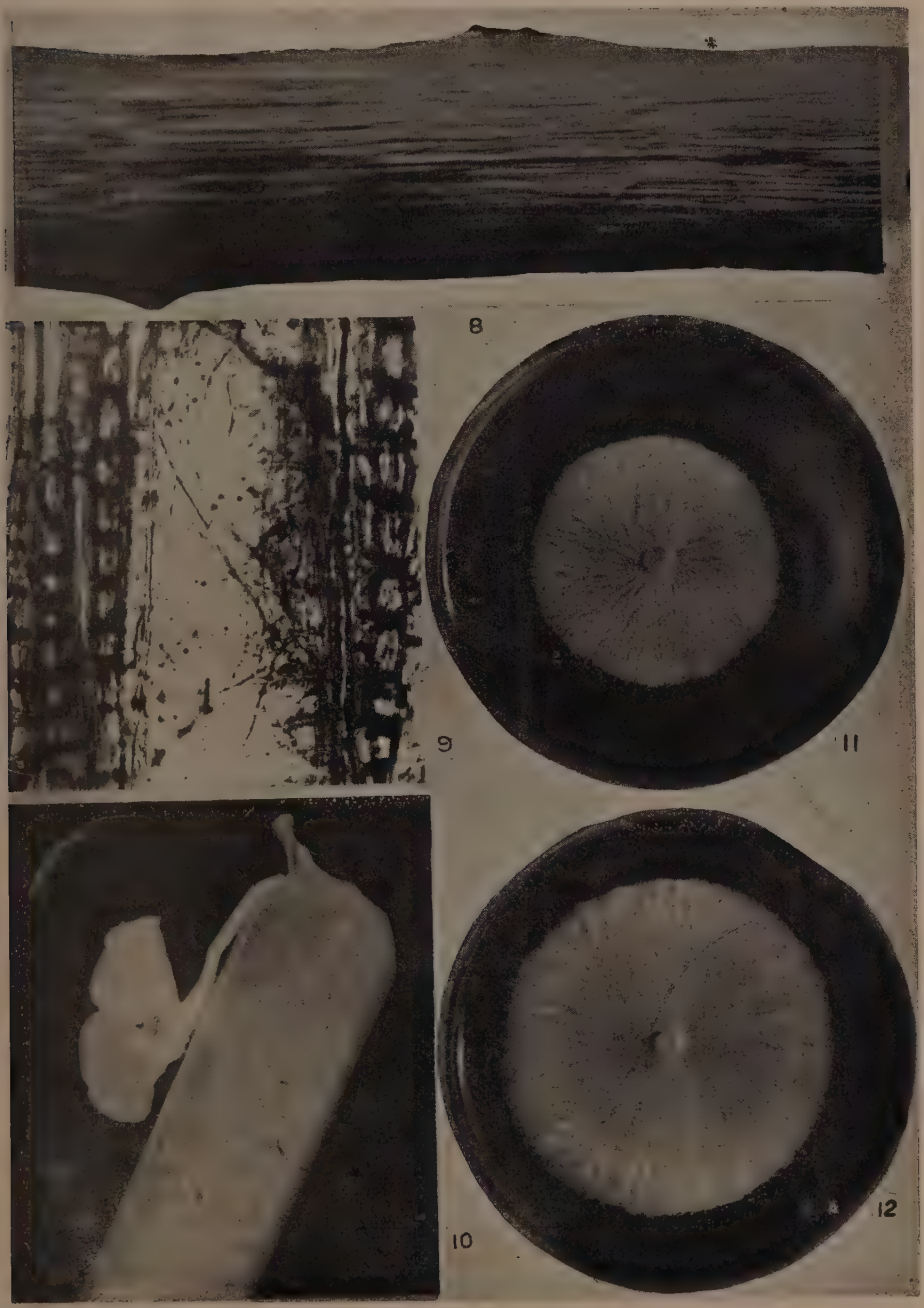


PLATE III

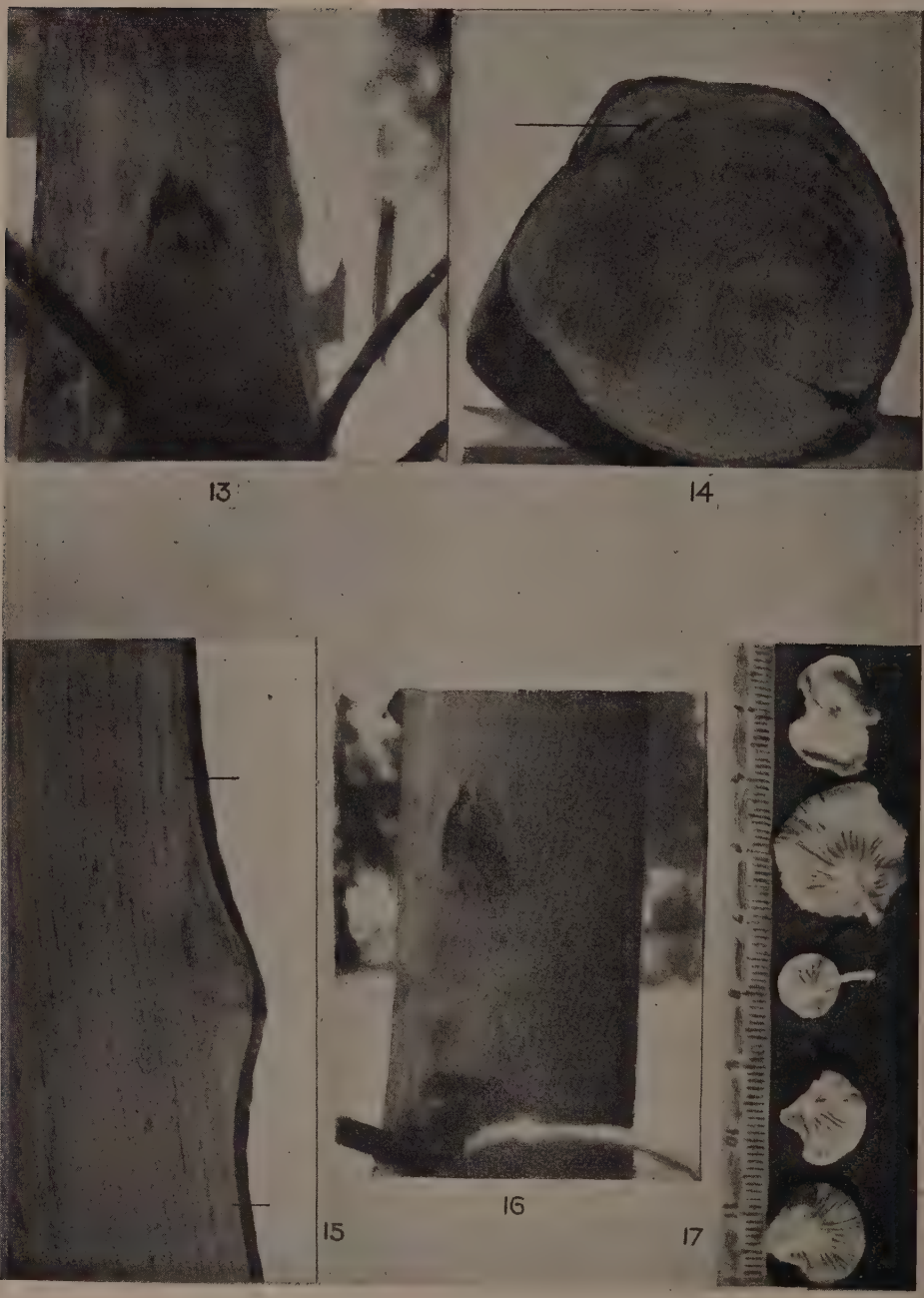
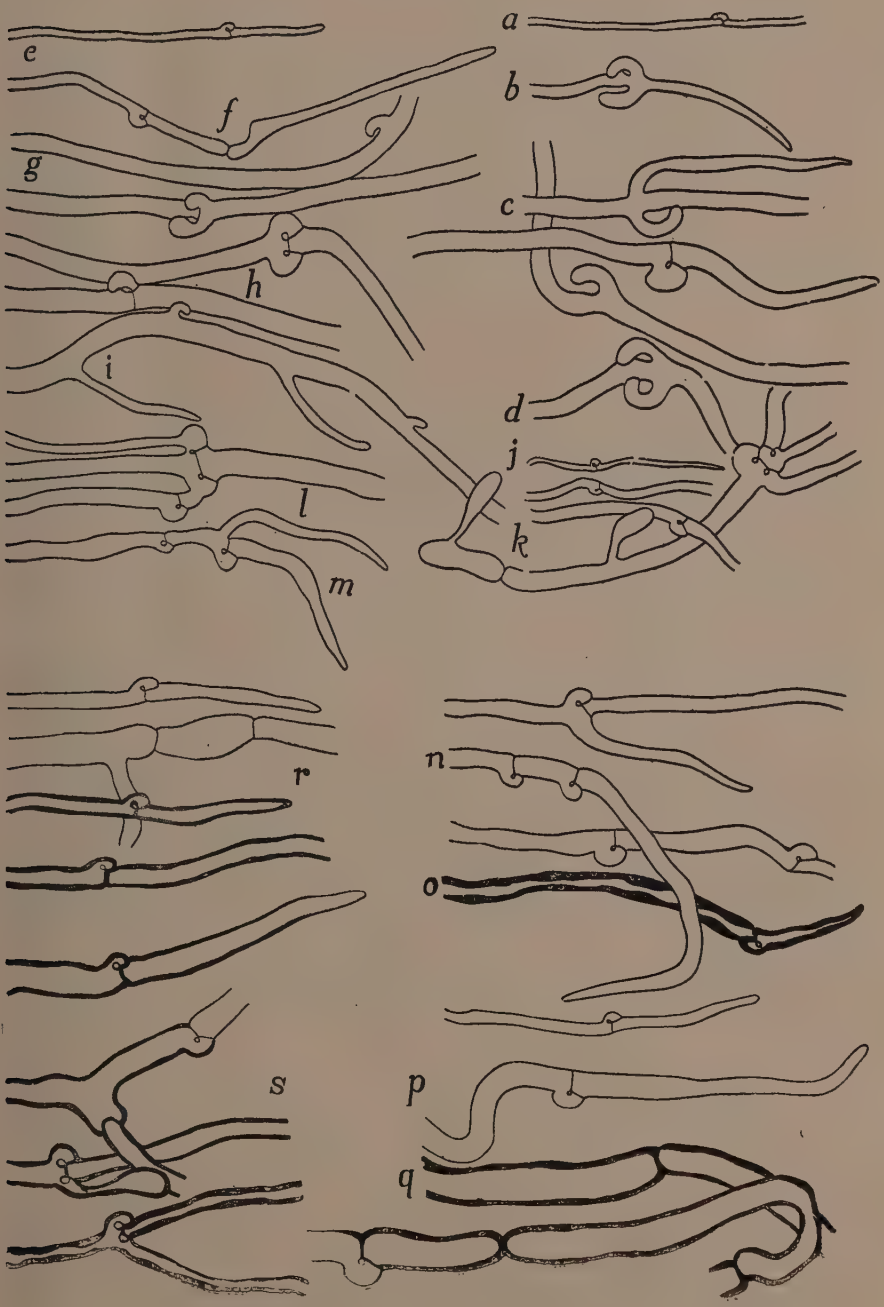


PLATE IV



NOTES ON SOME FUNGI FROM SOUTH INDIA—IV

T. S. RAMAKRISHNAN AND N. V. SUNDARAM

(Accepted for publication, December 20, 1954)

Woroninella umbilicata (B. & Br.) Petch

Syn. *Aecidium umbilicatum* B. & Br.

A. cajani Petch

Petch, T., *Trop. Agriculturist*, 1, 162, 1918.

On living leaves, stems and pods of *Cajanus cajan* (L.) Millsp., (Papilionatae), Cinchona (Anamalais), 22-4-54, T. S. Ramakrishnan and N. V. Sundaram.

This fungus has not been recorded so far from South India. Severe infection of the host was prevalent on the Anamalais. Numerous reddish orange galls were developed on the leaves, stem and pods. These were isolated or crowded together becoming cupulate on dehiscence. One or more bright reddish orange sori were found in each gall and these measured 230 to 500μ. The sporangia were thin-walled and measured 22×19μ (15—25×12 - 22). Hypnospores were not found.

Petch (1909) described *Aecidium cajani* on *C. cajan* (*C. indicus*) from Ceylon. Berkeley and Broome (1875) created *A. umbilicatum* on *Phaseolus grahamianus* and *A. cajani* was considered identical with this (Petch, 1912). In 1918 Petch revised *A. umbilicatum* as *W. umbilicata*. Petch and Bisby (1950) state that apparently the same species is present on *P. calcaratus*, *C. cajan*, *Atylosia rugosa*, etc. Gaumann did not accept the generic name *Woroninella* and considered it as synonymous with *Synchytrium*. If this is accepted it will be necessary to revise the name as *S. umbilicatum*. *W. umbilicata* is described as having hyaline spores whereas the fungus on *C. cajan* has bright orange sporangia.

Gupta and Sinha (1951) described *Synchytrium phaseoli-radiati* as occurring on *Phaseolus radiatus*, *P. mungo*, *Cajanus cajan* and *Crotalaria juncea* from Agra but this species produces only hypnospores whereas the fungus under study forms only sporangia and agrees with *W. umbilicata*.

Peronospora obovata Bonorden

Saccardo, P. A., *Syll. Fung.* 7, 252, 1888.

On living leaves and stems of *Spergula arvensis* L. (Caryophyllaceae), Nanjanad (Nilgiris), 19-5-54, T. S. Ramakrishnan.

Spergula arvensis is a very troublesome weed on the Nilgiris. In the month of May 1954 after the receipt of showers a blight of this weed was prevalent at the Agricultural Research Station, Nanjanad. The stem and leaves were yellowish and on these portions a downy growth of the conidiophores and conidia was present. The conidiophores were stout and emerged through the stomata in groups. They were repeatedly branched ending in pairs of ultimate branchlets. The conidia were elliptic to obovate and when mature assumed a smoky colour. Old conidiophores had sometimes one or two partition walls in the main axis. The spread of this disease on the spurry may be useful in keeping it in check. Several of the affected plants had dried up as a result of infection.

Elsinoe culleniae sp. nov.

Infection spots minute, circular or irregular, dark brown, often several spots become confluent to develop into irregular branched lichenoid scabby areas, epiphyllous, sometimes occupying large areas of the leaves ; ascomata intra-epidermal, olive brown ; asci in one or two layers, embedded in the stroma, obovate, double-walled, hyaline. $28\times25\mu$ ($22-38\times19-27$) ; ascospores 4, elliptical, thin-walled, 4-celled, later becoming muriform, $19\times8\mu$ ($15-22\times6-9$).

Infectionis maculae minutae, circulares vel irregulares, fusce brunneae, saepe plures confluentes in areas lichenoideas, crustaceas, irregulares, ramosas, epiphyllae, saepe maximam foliorum paginam occupantes ; ascomata intraepidermalia, olivaceo-brunnea ; asci in simplici vel duplici serie, stromati infixi, obovati, pariete duplici praediti, hyalini, $28\times25\mu$ ($22-38\times19-27$) ; ascosporae 4, ellipticae, parietibus tenuibus ornatae, 4-cellulatae, demum muriformes evadentes, $19\times8\mu$($15-22\times6-9$).

On living leaves of *Cullenia excelsa* W. (Malvaceae), Kadamparai (Anamalais), 22-4-54, T. S. Ramakrishnan and N. V. Sundaram.

Numerous scabby spots are found on the upper surface of the leaves which later become confluent. In the final stages a major portion of the surface is covered by them. The general appearance and the branching nature of the scabbed area bear a close resemblance to the growth of epiphytic algae or lichens. The stroma is developed intra-epidermally. This leads to the raising of the upper epidermal wall covering the stroma. The asci are formed in one or more layers embedded in the stroma.

Homostegia symploci Racib.

Theissen, F. and Sydow, H., *Ann. Myc. 13*, 606, 1915.

On living leaves of *Symplocos spicata* Roxb. (Symplocaceae), Pannaikadu (Pulneys), 2-4-54, N. V. Sundaram.

Hypertrophied dark brown spots are found on the leaves. The perithecia are deep-seated in the tissues and are present on both the sides. They are embeded in stromata. Each stroma may be uni or biloculate. The asci are cylindric with 8 brown spores. Paraphyses

are present. The ascospores are spindle shaped, 4-celled, tapering towards the two ends and with a prominent constriction in the centre at the middle septum. This fungus agrees closely with *H. symploci* recorded on this host genus and is identified as such.

Laestadia alternantherae sp. nov.

Spots roundish, light brown, amphigenous; perithecia isolated, rarely united in pairs, subepidermal, subglobose, amphigenous, ostiolate, 79—124×72—118μ; periphyses present; asci clavate, non-stipitate, hyaline, 46×10.5μ (36—58×9—12); ascospores irregularly biseriate, 8, hyaline to sub hyaline, oblong, straight or sometimes curved, 12×6.5μ (9—15×6—7.5).

Maculae plus minus circulares, pallide brunneae, amphigenae; perithecia separatim, raro binatim aggregata, subepidermalia, subglobosa, amphigena, ostiolata, 79—124×72—118μ; paraphyses adsunt; asci clavati, non-stipitati, hyalini, 46×10.5μ (36—58×9—12); ascosporae irregulariter biseriatae, 8, hyalinae vel subhyalinae, oblongae, rectae vel nonnumquam curvatae, 12×6.5μ (9—15×6—7.5).

On living leaves of *Alternanthera sessilis* R. Br. (Amaranthaceae), Cinchona (Anamalais), 20-4-54. T. S. Ramakrishnan and N. V. Sundaram.

Perithecia are distinct and globose with a dark membraneous covering. In each spot a number of perithecia are developed opening either on the upper or lower surfaces. Typical paraphyses were not observed.

Phyllachora flemingiae sp. nov.

Stromata black, isolated, conical, amphigenous, clypeate, shiny; loculus single, bounded on both sides by thick blackened stromatic tissues, ostiolate, 112—200μ in diam.; asci numerous, clavate, short stipitate, hyaline, 8-spored, 54×9μ (49—81×7—11); ascospores elliptic, monostichous or irregularly biseriate, hyaline, 10.5×7μ (9—12×6—7.5).

Stromata atra, separata, conica, amphigena, clypeata, nitentia; loculus unus, circumdatus ad apicem atque basim crassis stromatalibus textibus nigrescentibus, ostiolatus, 112—200μ diam.; asci plures, clavati, breves stipitati, hyalini, 8-spori, 54×9μ (49—81×7—11); ascosporae ellipticae, monostichae vel irregulariter biseriatae, hyalinae, 10.5×7μ (9—12×6—7.5).

On living leaflets of *Flemingia semialata* Roxb. (Papilionatae), Pannaikadu (Pulneys), 2-4-54, N. V. Sundaram.

Definite spots are not evident but the stromata occur distributed on both the surfaces of the leaflet in sparse groups. A thick band of black stromatoid tissue is present on the upper and the lower sides of the loculus. The entire thickness of the leaflet is occupied by the loculus and the stromatic tissue.

Physalospora jasminii sp. nov.

Spots irregular, amphigenous, light brown; perithecia amphigenous, appear as black dots, immersed in the tissues, ostiolate, 140—240×112—170μ; asci clavate, hyaline, thin-walled, 70×12μ (56—80×9—15), paraphysate; ascospores 8, monostichous, elliptical, hyaline, 12×9μ (10—15×6—10).

Maculae irregulares, amphigenae, pallide brunneae; perithecia amphigena, apparentia ut maculae nigrae, immersa in textus, ostiolata, 140—240×112—170μ; asci clavati, hyalini, tenuibus parietibus praediti, 70×12μ (56—80×9—15), paraphysati; ascosporae 8, monostichae, ellipticae, hyalinae, 12×9μ (10—15×6—10).

On living leaves of *Jasminum rigidum* Zenk. (Oleaceae), Pannaikadu (Pulneys), 2-4-54, N. V. Sundaram.

The spots are varying in size and are either marginal or otherwise. The perithecia appear as black dots. A black stromatic tissue is confined to the ostiolar region and is not found elsewhere. The perithecial wall is made up of 2 to 3 layers of brown cells.

Aecidium spilanthis sp. nov.

Rust spots circular, yellowish; pycnia not observed; aecia hypophyllous, in groups, cupulate, bright yellow, subepidermal, peridiate, 180—350×170—280μ; peridial cells polygonal, hyaline, thick-walled, strongly verrucose, 22—38×15—22μ; aeciospores catenulate; subglobose or polygonal, surface finely verruculose, with orange contents, 19×12μ (15—22×9—15).

Maculae circulares, luteolae; pycnia haud visa; aecia hypophylla, aggregata, cupulata, fulgide lutea, subepidermalia, peridiata, 180—350×170—280μ; cellulis peridialibus polygonalibus, hyalinis, crasse parietatis, fortiter verrucosis, 22—38×15—22μ; aeciosporae catenulatae, subglobosae vel polygonales, facie verruculosa, contentis aurantiacis, 19×12μ (15—22×9—15).

On living leaves of *Spilanthes acmella* Murr. (Compositae), Valparai (Anamalais), 21-4-54, T. S. Ramakrishnan and N. V. Sundaram.

Angiopsora cyrtococci sp. nov.

Rust spots brown, amphigenous, narrow, elongated; uredia amphigenous, sometimes arranged in lines, minute, yellowish orange when fresh, subepidermal, erumpent, crowded, with incurved paraphyses; paraphyses subhyaline, apices thickened irregularly, 27—42×12—17μ; urediospores subglobose to elliptic, yellowish brown, almost sessile, echinulate, pores not distinct, 22×19μ (19—28×16—22); telia subepidermal, dark brown, hypophyllous, mixed with uredia, long covered by the epidermis; teliospores one-celled, catenulate, in rows of 1-3, usually 2, angularly oblong, 24×12μ (15—34×9—16), wall sub-hyaline.

Maculae brunneae, amphigenae, angustae, elongatae; uredia amphigena, nonnumquam in lineas disposita, minuta, luteo-aurantiaca primo, subepidermalia, erumpentia, aggregata, paraphysibus incurvatis ornata; paraphyses subhyalinae, apicibus irregulariter crassis, 27—42×12—17μ; uredosporae subglobosae vel ellipticae, luteo-brunneae, fere sessiles, echinulatae, poris haud distinctis, 22×19μ (19—28×16—22); telia subepidermalia, fusce brunnea, hyphophylla, urediis intermixta, longa, epidermate operta; teliosporae semel cellulatae, catenulatae, dispositae in ordines 1—3, ut plurimum 2, angulariter oblongae, 25×12μ (15—34×9—16), parietibus subhyalinis.

On living leaves of *Cyrtococcum oxyphyllum* Stapf (Gramineae), Periakallar (Anamalais), 25-5-54, T. S. Ramakrishnan.

The rust is a typical *Angiopsora* with both uredia and telia. The uredia are prominent owing to the yellow colour of the sori which are arranged close togather in lines. The telia are darker and mixed with the uredia. Though the uredia have been observed on previcus occasions from the same locality the telia were noticed only now.

Cystopsora oleae Butl.

Butler E. J., *Ann. Mycol. 8*, 444, 1910.

On living leaves and stem of *Olea dioica* Roxb. (Oleaceae), Anamalais, 20-4-54, T. S. Ramakrishnan and N. V. Sundaram.

This rust is common in Burliar, Wynaad and Anamalais infecting the leaves and twigs of *Olea dioica* often producing witches broom like growths of clusters of shortened or diminitive shoots bearing aecia. It was observed that the rust was prevalent year after year on the same plant in the above localities. This led to the suspecion that the rust may survive from year to year in those plants by systemic infection. In order to determine whether systemic infection is present histological examination of the shoots of the diseased plants was made. Hyphae of the rust were found to permeate the tissues of the stem far remote from the point of production of witches brooms. The hyphae were hyaline and inter-cellular. Digitately branched haustoria were sent into the cells. From the distribution of the hyphae into the tissues it is inferred that the infection becomes systemic and leads to the successive production of malformed shoots year after year. Some regions of the infected shoots are swollen denoting the places from which witches brooms may develop latter.

Hamaspora longissima (Theum). Koern.

Koernicke, Fr., *Hedwigia, 16*, 23-24, 1877.

On living leaves of *Rubus lasiocarpus* Sm. (Rosaceae), Anamalais, 25-5-54, Nanjanad (Nilgiris), 29-5-54, T. S. Ramakrishnan.

Uredia are very minute, subepidermal, erumpent, bordered by cylindrical incurved hyaline paraphyses. The urediospores are globose or sub globose, sub hyaline to yellow, echinulate, germ pores not distinct and measure 25×19 μ (22—28×16—22). The telia are

hypophyllous, very prominent, in groups of filiform, ochraceous structures. Each telium originates sub-epidermally and later bursts through the epidermis. Marginal paraphyses, some times incurved, are present in the telia also. The pedicel of the teliospore is very long and hyaline. The teliospores are 2—5 septate with bright orange coloured contents and hyaline wall. They measure 197×19 μ (68—276×15—25). The terminal cell is usually hyaline and sharply pointed, sometimes being rostrate. The teliospores germinate readily producing a stout promycelium from each cell. The promycelium is 4-celled, curved and with orange coloured contents. A sterigma is produced from each cell of the promycelium on which a basidiospore is borne.

Phakopsora vignae (Bres.) Arth.

Arthur, J. C., *Bull. Torrey Bot. Club, 44*, 509, 1917.

On living leaflets of *Phaseolus lunatus* L. (Papilionatae), Kadamparai (Anamalais), 22-4-54, T. S. Ramakrishnan and N. V. Sundaram.

Reddish brown angular spots are developed on the leaflets. The uredia appear as small conical structures on the lower surface of these spots. Each uredium has a pseudoperidium formed partly of paraphyses and partly of hyphal cells. Through an apical pore in the roof of the sorus the spores are disseminated. The urediospores are almost sessile and are usually hyaline measuring 25×19μ (19—37×16—22). They appear as white powdery mass at the mouth of the sorus.

Phakopsora vignae has been recorded on *Phaseolus lunatus* outside India. Though only the uredial state is present it resembles closely the uredial state of *P. vignae* and the spore measurements are in agreement. Hence the rust is identified as *P. vignae*.

Puccinia hydrocotyles (Link) Cke.

Sydow, H. & P. *Monogr. Ured. 1*, 388, 1910.

On living leaves of *Hydrocotyle confierta* Wight (Umbelliferae) Agricultural Research Station, Nanjanad (Nilgiris), 29-5-54, T. S. Ramakrishnan.

The uredial state alone was prevalent on the leaves. The Sori were amphigenous but more common on the upper surface arranged singly and scattered, or in rings. The urediospores were of various shapes with cinnamon brown echinulate wall and 2 conspicuous germpores. The measurments of the spores *viz.*, 25×22 μ (22—31×19—25) agree with those of *P. hydrocotyles*. This rust has not been recorded from S. India.

Puccinia terminaliae (Ramak. T. S. & K.) Ramak. & Sund. nov. comb.

Syn : *Aecidium terminaliae* Ramak. T. S. & K. in *Proc. Ind. Acad. Sci. B. 27*, 43-44, 1948.

Rust spots on leaves amphigenous, with a distinct spherical woody gall in the centre, woody galls on branches also; pycnia amphigenous, intra and subepidermal, convex or spherical; aecia sunk in the woody galls, varying in number, cupulate hard-rimmed, measuring 670 – 910 μ high and 360—700 μ broad, peridiate; peridium ephemeral, made up of polygonal smooth-walled cells; aeciospores catenulate, polyhedral, with thick walls, one end sometimes thicker than the other, densely and prominently verruccose, measuring 42×24 μ (29—59$\times$19—29), deep reddish brown; uredia epiphyllous, in the middle of indefinite brown spots, sub-epidermal; urediospores pedicellate, pedicel hyaline, persistent, obovate or oblong, 33×25 μ (27—43$\times$22—28). wall lamellate with outer hyaline and inner reddish brown layers, thick, reticulately thickened, germpores 4, subequatorial; telia hypophyllous, subepidermal, dark brown; teliospores pedicellate (with a persistent hyaline pedicel up to 36 μ long, 9 μ broad), 2 celled, sub-globose to dumb-bell shaped, variously constricted at the septum, wall lamellate with an outer subhyaline and an inner reddish brown thickened layer, 52×27 μ (37—56$\times$22—31), sparsely and prominently echinulate, germpore one in each cell.

On living leaves of *Terminalia bellerica* Roxb. (Combrataceae), Valparai (Anamalais), 20-4-54, T.S. Ramakrishnan and N.V. Sundaram.

Ramakrishnan, T. S. and K. (1948) have described *A. terminaliae* on this host. Now the uredia and telia were also found associated with the *aecidium* on the same host. The pycnia exhibit variations with regard to the position. They are always formed on the hypertrophied tissues. They originate as subcuticular structures and later become intra epidermal. Such pycnia are hemispherical or convex but in others the pycnia are more deep seated becoming clearly subepidermal. It is rather interesting to find such variations in the disposition of a conservative fructification of the rust. The aecia have only ephemeral peridia. The aeciospores exhibit the presence of 2 lateral germpores. The urediospores are characterestic with a bilamellate wall reticulately thickened. The germpores are usually in a line but they occur either just below the middle or further down towards the pedicel. The teliospores possess a characterestic bilamellate wall with sparsely arranged prominent echinulations from the subhyaline outer wall. The constriction at the spetum is of varying depths being slight in some instances and very deep in others. Consequently the two cells become easily separated in some spores. The germ pore is apical in the upper cell and near the attachment of the pedical in the lower cell. The rust definitely belongs to the genus *Puccinia* though the lemellate wall may suggest other allied genera. The formation of the pycnia, aecia and uredia all point to the inclusion of the rust under *Puccinia*.

Ascochyta commiphorae sp. nov.

Spots amphigenous, light brown, of varying shapes, surrounded by a distinct dark brown margin, 1-3 mm across; pycnidia epiphyllous, subepidermal, ostiolate, appear as black dots on the surface; pycnidiospores elliptic or oblong, straight or slightly curved, one-septate, hyaline, $15\times6\mu$ (9—19$\times$4.5—7.5).

Maculae amphigenae, pallide brunneae, figurae variabilis, circumdatae margine fusce brunneo distincto, 1-3 mm. diam; pycnidia epiphylla, subepidermalia, ostiolata, apparentia ut maculae nigrae in superficie; pycnidiosporae elliptico-oblongae, rectae vel tenuiter curvatae, 1-septatae, hyalinae, $15\times6\mu$ ($9-19\times4.5-7.5$).

On living leaflets of *Commiphora caudata* Engl. (Burseraceae), Aduthurai (Tanjore), 28-4-54, T. S. Ramakrishnan.

Numerous distinct spots are formed on the leaflets which are at first dark brown but on drying turn lighter.

Cercosporella endecaphyllae sp. nov.

No definite infection spots, but white, powdery patches formed on the upper surface, internal hyphae hyaline; conidiophores fasciculate, emerging through the stomata, hyaline, septa absent $24-45\times4.5-6\mu$, flexuous; conidia obclavate,elongated, hyaline, septa indistinct, 3 to 5, $47\times3.5\mu$ ($27-78\times3-5$), tapering towards the apex.

Maculae infectionis haud definitae, sed albae, pulverulentae, areis claris efformatis in superficie superiore, hyphis internis hyalinis; conidiophori fasciculati, emergentes per stomata, hyalini, septo nullo ornati, $24-45\times4.5-6\mu$, flexuousi; conidia obclavata, elongata, hyalina, septis indistinctis, 3 to 5, $47\times3.5\mu$ ($27-78\times3-5$), fastigata ad apicem.

On living leaves of *Indigofera endecaphylla* Jacq. (Papilionatae), Kadambarai (Anamalais), 12-4-55, T. S. Ramakrishnan and N. V. Sundaram.

Cercosporina imperatae (Syd. H. & P.) Sawada

Sydow, H., *Ann. Mycol. 14*, 372, 1916.

On living leaves of *Imperata arundinaceae* Cyr. (Gramineae), Aduthurai (Tanjore), 28-4-54, T. S. Ramakrishnan.

Numerous confluent light yellowish spots are formed on the leaves. Studded on the surface of the spots are several minute black specks denoting the location of the conidiophores and conidia. The conidiophores come out in clusters through the stomata. They are septate, light brown and measure $25-50\times6-8\mu$. The conidia are obclavate and light brown measuring $27-45\times4-7\mu$ and have 3—4 septa.

Septoria delonicis sp. nov.

Pycnidia are isolated but in groups on small raised spots, amphigenous, black on the upper side, subepidermal, deep seated, ostiolate, $170-250\times140-195\mu$; pycnidiospores elongated, tapering towards the apex, 2-3 septate, $45\times4.5\mu$ ($36-54\times3-6$).

Pycnidia inter se distincta, sed aggregata in loca par vula elevata, amphigena nigra in superficie superiore, subepidermalia, alte infixa, ostiolata, $170-250\times140-195\mu$; pycnidiosporae elongatae, desinentes in 2-3 septatum apicem, $45\times4.5\mu$ ($36-54\times3-6$).

On living leaflets of *Delonix elata* Gamb. (Papilionatae), Coimbatore, 5-4-54, N. V. Sundaram.

On each leaflet several pycnidia are present. These are embeded in hypertrophied tissues which are slightly raised on both surfaces. On the upper surface black exudate collects at the mouth of the pycnidium giving it a characterestic colour.

Septoria erythrinae Ramak. T. S. & K.

Ramakrishnan, T. S. & K. *Proc. Ind. Acad. Sci.* B. *26*, 11, 1947.

On living leaves of *Erythrina indica* Lam., Coimbatore, 10-11-53, N. V. Sundaram.

This was previously recorded on *Erythrina* sp.

Septoria lycopersici Speg.

Saccardo, P. A., *Syll. Fung. 3*, 535, 1884.

On living leaves of *Lycopersicum esculentum* Mill. (Solanaceae), Cinchona (Anamalais), 20-4-54, T. S. Ramakrishnan and N. V. Sundaram; Coonoor, 23-6-54, T. S. Ramakrishnan.

Numerous roundish or irregular spots are visible on both sides of the leaves which lead to their general yellowing. In cases of severe infection the leaves dry up and the plants may succumb to the disease. Pycnidia are sub-epidermal and are oval or subglobose appearing as minute black dots on the spotted region. The spores are thin, filiform, flexuous, measuring $31-75\times2-5\mu$ with 2 to 5 septa.

Though the fungus has been recorded from Ceylon (Petch & Bisby 1950), it has not been reported from India.

We are indebted to Rev. Fr. H. Santhapau, S. J. of St. Xaviers' College, Bombay, for having kindly translated the diagnoses into latin. Our thanks are due to the Systematic Botanist and Professor of Botany, Agricultural College, Coimbatore for identifying some of the host plants.

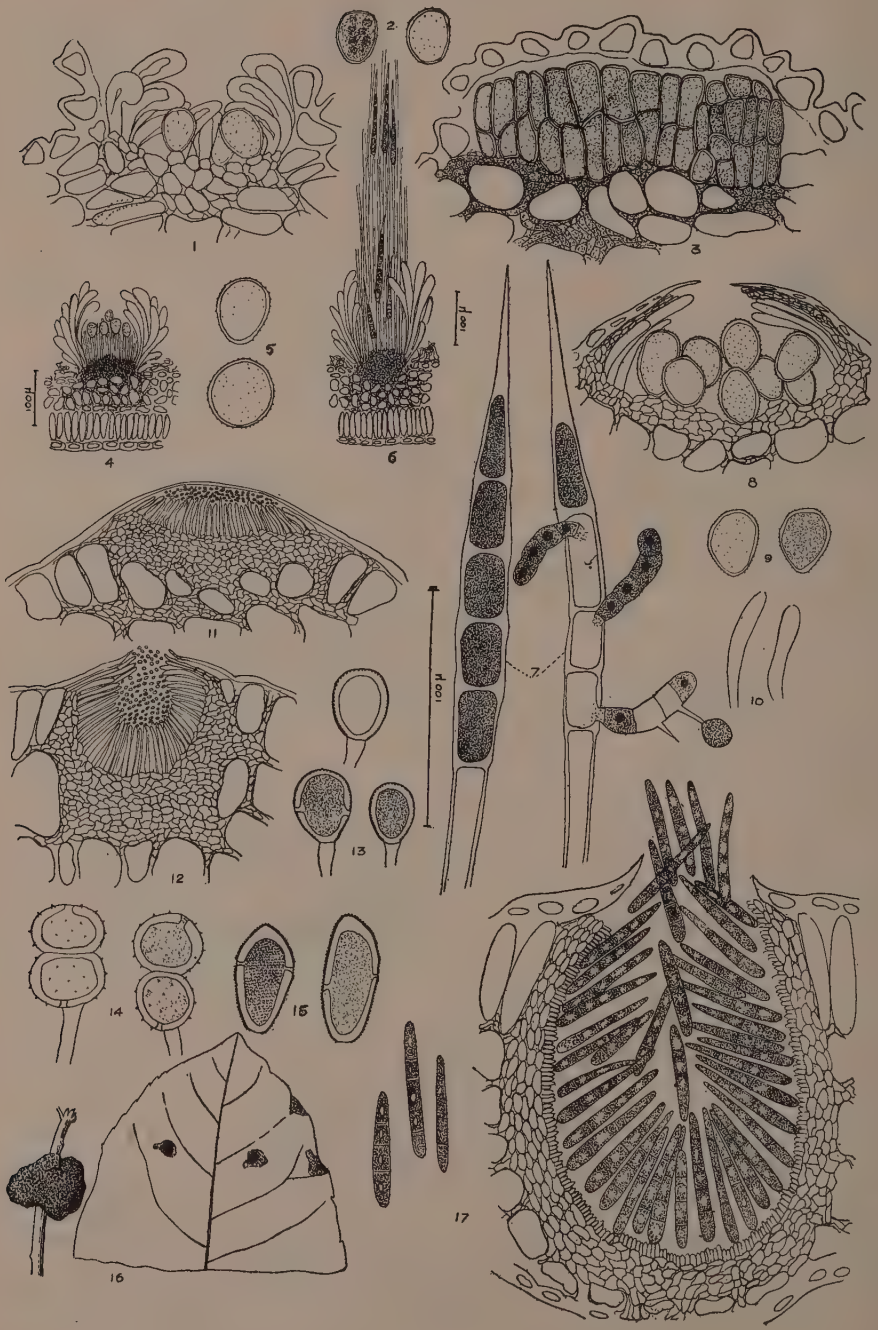
"Sankaram"
R. S. Puram
Coimbatore.

References

Berkeley, M. J. & Broome, C.E.	(1875)	*Jour. Linn. Soc. Bot.* (Lond.) **14**, 95-96.
Gupta, S. C. & Sinha, S.	(1951)	*Indian Phytopathology*. **4**, 7.
Petch, T.	(1909)	*Ann. Roy. Bot. Garden, Peradeniya*, **4**, 302.
Petch, T.	(1912)	*Ibid.* **5**, 256.
Petch, T.	(1918)	*Trop. Agriculturist*, **1**, 162.
Petch, T. & Bisby, G.R.	(1950)	The Fungi of Ceylon. Ceylon Government Press. pp. 18 & 71.

PLATE I

PLATE II



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RHIZOME AND ROOT ROT OF TURMERIC CAUSED BY *PYTHIUM GRAMINICOLUM* SUB.

T. S. RAMAKRISHNAN AND C. K. SOWMINI

(Accepted for publication, December 20, 1954)

INTRODUCTION

In recent years a new disease of turmeric (*Curcuma longa* L.) has been observed to be prevalent in Krishna district (Andhra State), and in Tiruchirapalli and Coimbatore districts (Madras State). The leaves of the diseased plants exhibit gradual drying along the margins. In course of time all the leaves dry up. The base of the aerial shoots may sometimes appear water-soaked and soft to touch. The symptoms may be seen in isolated plants or may involve several adjacent clumps resulting in diseased patches. The root system of the affected plants is very much reduced and consists of a few discoloured and rotten roots. In advanced cases the infection progresses into the rhizomes which become soft, rotten and poorly developed. The yield of rhizomes from such plants is practically nil.

MORPHOLOGY OF THE PATHOGEN

Microscopic examination of the affected roots, rhizomes or basal portions of the aerial shoots revealed the presence of hyaline non-septate hyphae permeating the tissues, both between and across the cells. Reproductive bodies were, however, not present. As the infection progressed the internal tissuses disintegrated and the outer skin was depressed. The bright orange colour of the tissues changed to different shades of brown.

When bits of the rhizome were removed aseptically from the advancing margin of the infected portion and transferred to oat agar, fluffy growths of a species of *Pythium* were obtained in all the plates. The isolate was purified by transferring single hyphal tips from cultures on plain agar to oat agar. This medium was found to be quite suitable for the growth of the isolate. It grew quickly and filled the Petri dishes in less than 48 hours after inoculation. A luxuriant growth of white aerial mycelium occurred. The hyphae were 3 to 8 μ in diameter and non-septate but septation was evident in the hyphae in the older portions of the culture. Asexual reproductive bodies were produced in abundance only when bits of culture were floated in water. These consisted of swollen lobulate sporangia filled with granular protoplasm and formed either terminally or as intercalary structures. Besides these, spherical thin-walled bodies were also produced either in the middle of the hyphae or terminally. These germinated by the development of germ tubes, or in other words, behaved as conidia.

Sexual reproduction was abundant on oat agar but was either absent or rare on Richards agar. The oogonia were spherical, smooth, thin-walled, terminal or intercalary and measured on an average 28μ in diameter (the range being 21 to 38 μ). The antherida were normally

monoclinous and one to six were observed to be attached to a single oogonium. The oospores in most cases filled the oogonia. They were spherical with a slightly thickened wall which assumed a brownish colour with age. The mean diameter of the oospores was 24 μ (the range being 17 to 34 μ).

PATHOGENICITY

Inoculations were carried out with pure cultures of the isolate to determine its pathogenicity on turmeric and its host range. In the first instance fresh rhizomes of turmeric bearing healthy roots were washed in 0.1 percent mercuric chloride solution for one minute followed by several changes of distilled water. These were kept in sterilised moist chambers and inoculated with the fungus. The roots were completely rotten in the course of one week and the infection had progressed into the rhizomes. The controls were quite normal without any sign of discolouration or rotting.

In the second series, inoculations were carried out on two months, old healthy turmeric plants (10 in number) grown in pots. The soil round the plants was mixed with the culture of the fungus on sand-oats medium. Marginal drying or wilting of the leaves became evident in 10 days and all the inoculated plants died down in 18 days. On examination it was found that the roots had decayed and the rhizomes and base of the shoots had softened with a water-soaked appearance. Hyphae of the pathogen were present in the affected portions. The control plants were healthy. The pathogenicity of the isolate on turmeric was thus established.

Other crop plants known to be parasitised by *Pythium* were next included in the studies to determine the host range of the isolate. The results are recorded below.

TABLE 1

Results of infection studies

Plant	Part inoculated	No. of plants inoculated	No. of plants infected	Remarks
Zingiber officinale	Collar region of aerial shoots	10	—	No infection
Nicotiana tabacum	Collar region and roots	10	—	do
Sorghum vulgare	do	10	9	The seedlings wilted and died down
Triticum vulgare	do	10	10	do
Zea mays	do	10	10	do
Hordeum vulgare	do	10	10	do
Avena sativa	do	10	10	do
Maranta arundinacea	Roots	10	—	No infection
Gossypium hirsutum	Collar and roots	10	—	do

The control plants of the different hosts remained healthy throughout. The isolate was found to be pathogenic to the seedlings of all the cereals included in the trials but not to the other plants. The collapse of the seedlings was brought about in 6 to 12 days.

Cultural & Other Studies of the Pathogen

The growth of fungi on synthetic media is influenced by the reaction of the substrate. Most of them thrive well on a slightly acidic medium. Garrett (1944) has quoted from Arrhenius that root rot of beet root caused by *P. debaryanum*, is favoured by acid soils. While Middleton (1943) found that in some of the isolates of *Pythium* he studied, oogonia in media above pH6 were often abortive. In order to determine the optimum reaction of the medium favourable for the growth of this isolate it was grown on oat agar adjusted to reactions varying from pH 3 to 9. The diameter of the growth on solid media is not always a reliable guide for the assessment of the amount of growth of fungi as this does not take into consideration the thickness of the growth. Therefore gravimetric estimation of the mycelial mats was also made by growing the fungus in Richards solution adjusted to different pH values. The data obtained are given below.

Table 2

Growth of the fungus on media of different pH values

Reaction of medium	pH						
	3	4	5	6	7	8	9
Diameter of growth in 24 hours on oat agar in mm.	7	8.3	32.8	36.8	33.0	30.8	32
Weight of fungal mat in Richards solution in 12 days in mgm.	270.9	311.3	316.5	316.8	403.7	566.3	272.7

The results indicate that the fungus can grow on media over a wide range of pH values. The Petri dishes were completely filled in all the media having pH values of 5 to 9 in 48 hours. The growth on oat agar of pH values 3 and 4 was very slow and poor. No oospores were produced on these. But they were formed in abundance on media having reactions of pH 6 to 9. Estimation of the intensity of oospore formation was made by cutting out discs of media of equal diameter (2 mm.) from the same region of growth in all cases and counting the number of oospores under the low power of the microscope with the help of Ehrlich's haemocytometer. Five readings were taken for each disc and the average determined. On the medium having a reaction of pH 5 the number of oospores was only half of what was

observed in pH 6 to 9. The intensity of oospore production was of the same magnitude between pH 6 and 9. The weight of the fungus mat in Richards solution was found to be the highest at pH 8. It is seen from the above that the fungus can tolerate a certain amount of acidity and alkalinity though the maximum growth was obtained at pH8 in Richards solution, *i. e.* in the alkaline range.

Vanterpool (1940) found that 'browning root rot' of wheat in Canada caused by *Pythium arrhenomanes* was favoured by unbalanced application of phosphorus and nitrogen and that the application of phosphatic manures brought down infection. This is attributed to the improvement of the root system of the plants. He also observed that farmyard manure gave adequate control of this disease compared to that obtained by phosphatic manures and has recommended the application of farm yard manure as a control measure. The clump rot of cardamoms prevalent in South India and attributed to *P. vexans* and *P. aphanidermatum* is kept in check by the application of superphosphate or ammonium phosphate to the soil (Ramakrishnan 1949). Root rots of rice and sugar-cane are also known to be favoured by excess of nitrogen. The influence of varying proportions of potassium nitrate and potassium phosphate in Richards medium on the growth of this fungus was determined both on solid media and in liquid cultures. The results are recorded below.

TABLE 3

Growth of the pathogen on Richards medium with different quantities of potassium nitrate and potassium phosphate.

KNO_3 gm./litre	12.5	10.0	7.5	5.0	2.5
KH_2PO_4 gm./litre	2.5	5.0	7.5	10.0	12.5
Average diameter of growth in 48 hours in mm.	46.5	45.5	47	58.5	53.5
Average weight of fungus mat at the end of 7 days in mgm.	390	420	386	353	347

The best growth as judged by the weight of the mycelial mat is obtained when the proportion of the components is maintained as in the normal Richards medium. There is decrease in growth when the proportion is modified but it is only to a limited extent. The growth of the fungus is not depressed appreciably by increasing the phosphate content of the medium.

These studies were followed up by observations on the effect of the application of ammonium sulphate and superphosphate to the soil on the intensity of infection. Garden soil was placed in glazed

pots and sterilized at 20 lb. pressure for two hours. Calculated quantities of ammonium sulphate or superphosphate to provide 2 cwt. or 1 cwt. per acre respectively were added to the pots and thoroughly mixed with the soil. Control pots without adding these manures were also maintained. Healthy rhizomes of turmeric were planted in the pots and after they had sprouted the plants were inoculated by placing the pure culture of the pathogen grown on sand-oats medium in the soil round the plants. Evidences of infection were obtained in the course of ten days. The results are shown below.

TABLE 4

Intensity of infection with different manures

Treatment	No. of plants used	No. of plants infected
Ammonium sulphate and inoculated with fungus	10	9
Ammonium sulphate but not inoculated	10	...
Superphosphate and inoculated with fungus	10	8
Superphosphate but not inoculated	10	...
Control (inoculated)	10	8
Control (uninoculated)	10	...

The pathogen was reisolated from the infected plants in all cases. It is evident from the above that the addition of superphosphate has not brought down the infection. It is therefore concluded that this method of control may not be applicable for combating this disease.

Foliar sprays of urea have been adopted to check root rot and die back of *Citrus* in South Africa and South India. To find out whether this chemical has any effect on the growth of this fungus it was added to oat meal agar in quantities to make up 0.1, 0.2 and 1 per cent of the media. The fungus did not grow in the plates containing 1.0 and 0.2 per cent of urea. At 0.1 per cent concentration the growth was slower and submerged while in the control quicker growth was obtained with plenty of fluffy aerial mycelium. Oospores were not formed in the media to which urea had been added (up to 8 days) while they were formed in abundance in the control plates. Urea appears to have an inhibitory action on the fungus at the concentrations used and arrests the development of reproductive bodies.

To determine the effect of this chemical on the survival of the fungus in the soil, urea was added to sterilised soils in pots in different

quantities to make 0.1, 0.2, and 1.0 per cent concentrations. Later the soil was inoculated with the culture of the fungus in sand-oats medium. The pots were kept in the green house and the soil in the pots was maintained in a moist condition (35 per cent of the moisture holding capacity of the soil). Isolations of the fungus were made from the inoculated soil at intervals of a fortnight. The fungus could not be isolated from the soil containing 1.0 per cent urea after 45 days. In the soil containing 0.2 per cent urea the fungus remained viable for 165 days after which it could not be isolated. From the soil containing 0.1 per cent urea and in the control the fungus could be isolated up to 290 days when the experiment was discontinued. The survival of the fungus in the soil is observed to be adversely affected by the higher concentrations of urea. It is also seen that the fungus remains viable in the soil for long periods.

Identity of the Pathogen

The lobulate nature of the sporangia suggests that the isolate is closely allied to *P. graminicolum* or related species. The presence of spherical 'conidia' brings it nearer to the former species. The attachment of several antheridia to each oogonium and the measurements of the oogonia and oospores go to confirm this identity. Subramaniam (1928) who erected the species observed that it caused root rot of wheat. The inoculation experiments with the isolate under study have shown that besides turmeric it infects cereals including wheat. For these reasons the fungus is identified as *P graminicolum.*

Park (1934) has recorded a rhizome rot of turmeric in Ceylon caused by *P. aphanidermatum.* Middleton (1943) has reported that *Curcuma longa* is susceptible to *P. graminicolum, P. aphanidermatum* and *P.* sp. However there does not seem to be any record of this disease from India.

Control

The disease is at present confined to particular localities. The pathogen is mainly soil-borne. Hence all attempts to control infection must aim at sterlizing the soil. With this object in view the soil in small plots was artificially inoculated with the pure culture of the pathogen and then drenched with Cheshunt compound or 0.1 per cent solution of Ceresan (wet) at one gallon per square yard. Control plots without any fungicidal treatment were also maintained. Healthy rhizomes were planted in the different plots. There was no incidence of the disease in the treated plots while 70 per cent of the plants in the control plots became affected.

Based on these results it is recommended that for the control of the disease in the field, drenching the soil with either of the above fungicides can be adopted with advantage. Under field conditions, the affected plants should be eradicated and the soil round the neighbouring healthy ones should be treated as a preventive measure.

SUMMARY

A root and rhizome rot of turmeric is prevalent in Andhra and Madras States. The causal organism is *Pythium graminicolum* which infects the roots and rhizomes. Application of superphosphate to the soil does not keep down the intensity of infection. Eradication of the diseased clumps and drenching the soil with fungicides are recommended for the control of the disease.

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REFERENCES

Anonymous (1936) A list of the diseases of cultivated plants in Ceylon. *Dept. Agric. Ceylon, Bull.* 88: 1-47.

Garrett, S.D. (1944) Root disease fungi. Chronica Botanica Book Company, p. 47.

Park, M. (1934) Rept. on the work of the Myc. division. *Adm. Rept. Dir. Agric. Ceylon* 1933: 126-133.

Middleton, J. T. (1943) The taxonomy, Host range and Geographic distribution of the Genus *Pythium*. *Mem. Torr. Bot. Cl.* 20: pp. 1-171.

Ramakrishnan, T.S. (1943) The occurrence of Pythium vexans de B. in S. India. *Indian Phytopathology* 2: 27-30.

Vanterpool, T.C. (1940) Present knowledge of browning root rot of wheat with special reference to its control. *Sci. Agric.* 20: 735-749.

Subramanian, L. S. (1928) Root rot and sclerotial diseases of wheat. *Agric. Res. Inst. Pusa, Bull.* 177: 1-17.

PLATE I

1. Diseased turmeric plants. (Top)
2. Results of artificial inoculation. (Bottom)

PLATE I

COMPARATIVE STUDY OF SPECIES OF *XANTHOMONAS* PARASITISING LEGUMINOUS PLANTS IN INDIA

V. V. Bhatt and M. K. Patel

(Accepted for publication December 20, 1954)

Introduction

Leguminosae contains a large number of genera distributed in tropics with humid conditions and numerous species are found parasitised by phytopathogenic bacteria. Elliott (1951) records 2 species of *Agrobacterium*, 3 of *Corynebacterium*, 17 of *Pseudomonas* and 6 of *Xanthomonas* producing wide range of symptoms, like galls, wilt, leaf spots, etc., on 60 species distributed over 26 genera of Leguminosae. Patel and his co-workers have recorded 38 phytopathogenic bacteria from India, 34 of which belong to the genus *Xanthomonas*. It is also interesting to note that 16 of them incite diseases on members of Leguminosae only. Burkholder (1930) studied the bacterial diseases of beans including causal organisms belonging to 3 genera. Warnham (1948) and Patel, Dhande and Kulkarni (1951) studied several species of *Xanthomonas*, but so far no detailed comparative study of *Xanthomonas* species exclusively parasitising Leguminosae has been made. It was, therefore, thought worthwhile to undertake their comparative study with reference to nutrition derived from a particular group of plants of somewhat similar floral structure.

Source of Cultures

The pathogenic cultures used in the present study, as given in Table 1, were isolated from plants found in Bombay State and maintained on potato dextrose agar. It was interesting to note that *Xanthomonas vignicola*, *X. desmodii*, *X. alfalfae*, etc., isolated in 1946, 1947, and 1948 respectively and subcultured on a suitable medium at regular intervals were as pathogenic as *X. erythrinae* and *X. cyamopsidis* isolated in 1951 and 1952 respectively.

Materials and Methods

Unless otherwise mentioned, all the experiments were carried out at 30°C. on neutral media. Inoculations with standard loop were made from 48 hrs. old cultures grown in peptone dextrose liquid medium. In a liquid medium, 3 standard loops were transferred while a loop was drawn on agar medium. All the experiments were duplicated and repeated.

Symptoms

All the species of *Xanthomonas* parasitising members of Leguminosae in the present study incite spotting of leaves and canker on

injured tender shoots. The wilt symptoms as produced by *X. campestris* in *Brassica oleracea* (Cruciferae) and *X. uppali* in *Ipomoea muricata* (Convolvulaceae) could not be induced by artificial infection in Leguminosae. Bhide (1949) obtained stomatal invasion of *X. campestris* by using sodium oleate, but failed to observe wilt through stomatal invasion. Thus, the question arises as to why the wilt character is really specific for certain *Xanthomonas* species, otherwise undifferentiated, and that wilt is incited only in plants with hydathodes.

Morphology and Staining Reactions

Since Dowson (1939) described the genus *Xanthomonas* fully, no attempts were made for a detailed study.

Motility.—Motility by one polar flagellum was confirmed by using the method suggested by Patel, Kulkarni and Gaekwad (1950).

Gram reaction.—All the young and old cultures when stained by Hucker modification, Kopeloff's and Beerman's modified methods, consistently gave Gram negative reactions.

Capsule stain.—All the cultures, when stained by Hiss's method, were found to be capsulated.

Influence of Temperatare on Growth

All the cultures inoculated on potato dextrose agar slants and incubated for 4 days showed slight to fair growth at 10°, 15°, 20° and 35°, good to excellent at 25° and 30° and no growth at 0°, 5°, 40°, and 45°C. However, when those previously incubated at 0°, 5° and 40° were transferred at 30°C., growth was observed showing that the organisms were not killed.

The thermal death point of all the organisms lies near about 51°C.

Influence of Hydrogen-ion Concentration on Growth

Wolf and Shunk (1921) report that the hydrogen-ion alone does not inhibit growth and that different acids with the same hydrogen-ion concentrations do not exert the same influence in inhibiting cell multiplication. Berridge (1924) observed that the bacterial plant pathogens of *Pseudomonas* group seem to possess three associated characters, *viz.*, (*i*) high acid agglutination points, (*ii*) limits of growth and (*iii*) capacity to produce alkali rapidly in the presence of nitrogenous food material, less marked in the non-sporing saprophytes. She also observed that the wilt producing bacteria were generally confined to vascular bundles because of high acid agglutination point.

Generally, H-ion concentration of plants lies between 4 and 8. Whether such a character delimits the species, an experiment was laid out wherein synthetic carbohydrate medium was adjusted to different pH by Beckman pH meter using 0.1 N, HCl and NaOH for adjustment. Observations made after 8 days showed slight growth

TABLE 1

Source of cultures

S. No.	Name of culture	Authority	Source	Host	Date
1	*Xanthomonas alfalfae*	(Riker, Jones & Davis) Dowson	G. W. Dhande	*Medicago sativa* L.	1948
2	*Xanthomonas desmodii-gangetici*	Uppal, Patel & Moniz	M. K. Patel	*Desmodium gangeticum* D.C.	1946
3	*Xanthomonas cassiae*	Kulkarni, Patel & Dhande	Y. S. Kulkarni	*Cassia tora* L.	1948
4	*Xanthomonas vignicola*	Burkholder	N. D. Diwan	*Vigna catjang* Walp.	1946
5	*Xanthomonas cyamopsidis*	Patel, Dhande & Kulkarni	G. W. Dhande	*Cyamopsis tetragonoloba* (L.) Taub.	1952
6	*Xanthomonas stizolobiicola*	Patel, Kulkarni & Dhande	Y. S. Kulkarni	*Stizolobium deeringianum* Bort.	1950
7	*Xanthomonas erythrinae*	Patel, Kulkarni & Thirumalachar	Y. S. Kulkarni	*Erythrina indica* Lam.	1951
8	*Xanthomonas desmodii*	Uppal & Patel	M. K. Patel	*Desmodium diffusum* D.C.	1947
9	*Xanthomonas phaseoli-sojense*	(Hedges) Dowson	V. V. Bhatt	*Soja max* (L.) Piper	1952
10	*Xanthomonas sesbaniae*	Patel, Kulkarni & Dhande	Y. S. Kulkarni	*Sesbania aegyptiaca* Prain.	1949
11	*Xanthomonas tamarindi*	Patel, Bhatt & Kulkarni	V. V. Bhatt	*Tamarindus indica* L.	1950
12	*Xanthomonas cajani*	Patel, Kulkarni & Abhyankar	Y. S. Kulkarni	*Cajanus cajan* Millsp.	1949

at 4.2 and 9.3; fair at 5.3; good at 6.1 and 8; excellent at 7; and nil at 1.1, 2.0, 3.1 and 10.1 pH.

Cultural Characters

All the cultures grew copiously, producing smooth, circular and raised growth with different shades of yellow colour on potato dextrose agar, nutrient dextrose agar, yeast-dextrose-chalk agar plates and also made good growth without pellicle in nutrient broth. In Czapek's medium, *Xanthomonas alfalfae*, *X. cassiae*, *X. cyamopsidis*, *X. sasbaniae* and *X. cajani* made good to excellent growth, whereas *X. desmodii-gangetici*, *X. vignicola*, *X. stizolobiicola*, *X. desmodii*, *X. erythrinae*, *X. phaseoli-sojense* and *X. tamarindi* made no appreciable growth.

It will thus be seen that the general cultural characters of these 12 *Xanthomonas* species are not sufficient for differentiation. Czapek's medium, however, required a detailed study for nitrogen requirement either from the inorganic or organic compounds.

Gross Biochemical Reactions

All the cultures liquefied gelatin in Frazier's agar plates and plain gelatin in tubes, equally hydrolysed starches, like corn, potato, pulse, wheat and soluble and digested casein with no apparent differences. Hydrogen sulphide was produced by all from peptone water, whereas the agar medium suggested by Vaughan and Levine (1936) was found unreliable, as also reported by Bhide (1948). None produced indol when tested with Gore's reagent and oxalic acid paper strips. A definite acid reaction as against the alkaline one reported by Bergey *et al.* (1948) was observed at the end of 3 days in *Xanthomonas alfalfae*, *X. cassiae*; *X. cyamopsidis*, *X. erythrinae*, *X. desmodii*, *X. phaseoli-sojense*, *X. tamarindi* and *X. cajani* in litmus milk which turned to pale roseline purple. while the rest produced cameo pink after 5 days. These colours did not change to blue, indicating that the organisms attacked lactose and glucose of milk only and not lactalbumen. At the end of 8 days, litmus was reduced completely with peptonisation of milk. No curdling was, however, observed even at the end of 15 days. Liquefaction of Loeffler's blood serum was very quick in the case of *Xanthomonas vignicola* and *X. stizolobiicola*, fairly quick in *X. desmodii-gangetici*, *X. cassiae*, *X. erythrinae*, *X. desmodii*, *X. sesbaniae*, *X. tamarindi* and *X. cajani*, while *X. alfalfae*, *X. cyamopsidis* and *X. phaseoli-sojense* could not liquefy it completely even at the end of 16 days. Even though such a differential growth is observed on Loeffler's, the test hardly delimits the species but does help in a tentative classification of species, as was reported by Patel, Dhande and Kulkarni (1951) into groups.

Production of Ammonia

Peptone water when inoculated and observations made after 5, 8, 12 and 15 days with freshly-prepared Nessler's reagent showed that

Xanthomonas alfalfae, *X. desmodii-gangetici*, *X. desmodii*, *X. phaseolisojense* and *X. sesbaniae* produced ammonia after 8 days; *X. cassiae*, *X. stizolobiicola*, *X. erythrinae* and *X. cajani* after 12 days; and *X. vignicola*, *X. cyamopsidis* and *X. tamarindi* after 15 days.

Levine (1933) recommends a medium containing peptone and potassium nitrate for this purpose. Since all the organisms produced ammonia from peptone, it was thought worthwhile to try a synthetic medium containing only a nitrate salt as a source of nitrogen for confirmation. Thus, the following medium was prepared.

Potassium or Sodium nitrate	1.0	gr.
Potassium chloride	0.2	gr.
Magnesium sulphate	0.2	gr.
Sucrose	5.0	gr.
Distilled water	1000	ml.

This neutral medium was inoculated with a 48-hour-old culture grown on potato dextrose agar to allow maximum growth. Tests for ammonia were carried out from 24 hours to 30 days.

It was observed that the same 5 cultures that grew well in Czapek's medium made good growth in this medium also, but produced no ammonia upto 30 days. Patel, Dhande and Kulkarni (1951) and Riker, Jones and Davis (1935) observed ammonia production by *Xanthomonas* spp. from nitrate in nutrient broth. Burkholder (1937), on the other hand, observed good growth of *X. geranii* in a synthetic nitrate medium with production of ammonia. The conclusion drawn by Patel *et al* (1951) and Riker *et al* (1935) cannot be accepted since the medium contained peptone and nitrate, the former only found to produce ammonia.

An interesting fact from chemical point of view is worth noting here. Reducing sugars, like dextrose, glucose, lactose, maltose etc., are generally employed in the preparation of media. When any such sugars or aldehydes, like formaldehyde, acetaldehyde, etc., in ammonia-free distilled water were tested with Nessler's reagent, initial yellow colour of the solution changed to slight brown, deep brown and lastly ashy to black colour. However, no such change in colour was observed with sucrose, a non-reducing sugar, in distilled water free from ammonia. This indicates that the use of reducing sugars or aldehydes in a medium used for ammonia test is not desirable. Burkholder (1937) who did not observe colour change in controlled tubes has not specified the method or the reagent used for detection of ammonia from synthetic nitrate medium containing glucose.

The negative results obtained with synthetic nitrate medium as suggested here can be accounted for in two ways. Either, ammonia produced from nitrate is neutralised by the acid produced from sucrose, or ammonia is not produced by the cultures utilising nitrogen from nitrate. For the former reason, many modifications like increase of nitrate salt to 0.5 per cent and decrease of sucrose to 0.2 per cent were made. However, the results were negative. A *Bacillus subtilis* strain from hay infusion showing strong nitrite production

gave no ammonia test even after 30 days even though excellent growth was observed.

Production of Nitrite

Patel, Dhande and Kulkarni (1951) state that the ability to produce nitrite from nitrate is normally restricted to species of *Bacterium* as a whole, and to half the species of *Pseudomonas*, but is rare in the species of *Xanthomonas*. Bergey *et al* (1948) record 56 *Xanthomonas* species, of which 12 are reported to reduce nitrate to nitrite. The importance of a medium to be used for such a test is well stressed by Conn and Breed (1919), who observed that a culture producing nitrite from nitrate in one medium but not in another should not be assigned to a group unless detailed study is carried out.

Thus, of the two media employed to test nitrite production, one contained 0.5 per cent of peptone and 0.02 per cent of potassium nitrate as suggested by Conn and Breed (1919) and the other was synthetic nitrate medium as employed in ammonia production test. Tests were carried out every 24 hours with *B. subtilis* as control.

It was observed that all the cultures in peptone nitrate broth and *Xanthomonas** and *X. cajani* along with *B. subtilis* in synthetic medium made good growth but *B. subtilis* only continued to give positive test from 24 hours to 30 days. This clearly shows that *Xanthomonas* species under study do not reduce nitrate to nitrite. Then what happens to nitrate salt, the sole supply of nitrogen for growth? Since ammonia and nitrite are not produced, it seems possible that the five organisms utilise nitrogen from nitrate in such a complex way that neither ammonia nor nitrite is produced or, at any rate, they are not traceable.

Utilisation of Carbon Compounds

It is now an established fact that the composition of a basal medium employed in the study of fermentation of carbon compounds plays an important role. Burkholder (1932), probably the first to study comparative fermentation of 9 organisms commonly known as *campestris* group, inferred that, except for the production of acid or acid and gas from nutrient broth containing a carbon compound, the results are negative when hydrogen-ion concentration of the medium remains stationary (7 pH) or alkaline. Thus, he discarded the basal medium containing peptone and recommended a modified medium of Ayers, Rupp and Johnson (Manual, 1945). In the present study also, the above modifed medium with 1 per cent of carbon compounds and 0.1 per cent of organic acids was employed.

Observations made after the usual interval conclusively showed that these 12 *Xanthomonas* spp. equally utilised dextrose, levulose, galactose, arabinose, rhamnose, xylose, lactose, maltose, sucrose and starch making good to excellent growth with acid production. On the other hand, salicin, cellulose and benzoic, formic, oxalic, salicylic,

* *X. alfalfae, X. cassiae, X. cyamopsidis, X. sesbaniae.*

tannic and tartaric acids did not support the growth. In acetic, citric and lactic acids, the organisms made fair to good growth with alkaline reaction. *Xanthomonas alfalfae* utilized tri-saccharide, like raffinose, and alcohols, like dulcitol, glycerol and mannitol very well with acid production. On the other hand, *X. desmodii-gangetici* and *X. tamarindi* produced fair to no growth with very little change in pH, while the rest showed varied growth and pH reaction. In Table 2, an attempt has been made to classify them on the basis of their utilising these compounds.

From Table 2, it is clear that there exist 3 groups of 7 organisms, *i. e.*, *Xanthomonas vignicola* and *X. stizolobiicola* forming group 1, *X. desmodii*, *X. phaseoli-sojense* and *X. sesbaniae* forming group 2 and *X. desmodii-gangetici* and *X. tamarindi* forming group 3, which are indistinguishable from one another in the same group. The other 5 organisms are easily distinguishable from one another and also from the 3 groups mentioned above.

Effect of Amounts of Nitrogen on Growth

Vaughan (1943) observed differential growth of *Phytomonas solanacearum* on media containing different concerntrations of nitrogen in the form of plant sap or nitrate. It was, therefore, thought worthwhile to see the effect of different concentrations of nitrogen on growth using it in the form of asparagine and potassium nitrate ranging from 14 to 1483 p.p.m. Observations made 8 days after inoculation showed that all grew well in asparagine, whereas only 5 grew well in nitrate.

Utilisation of Nitrogenous Compounds

Micro-organisms are, as a rule, unable to grow in culture media not containing suitable nitrogen and carbon compounds. Some possess no discriminating tendency in their use of such compounds, whereas others are specific in their requirements, a phenomenon useful in distinguishing genera or species. Differential utilisation of carbon compounds had been observed by Burkholder (1932) and also in the present work. There are, however, more instances wherein a genus or species could be differentiated by growth characters and by nitrogen utilisation rather than by its ability to utilise carbon.

Kent (1942) studying the sources of nitrogen utilised by peritrichous bacterial plant pathogens as a possible adjunct to their classification could show that *Bacillus carotovorus*, *B. phytophthorus* and *B. aroideae* utilised nitrogen in the organic form. Of the 2 cultures designated as *B. lathyri*, one could utilise nitrogen from nitrate or ammonia, whereas the other could utilise only ammonia-nitrogen in addition to the organic nitrogen. Ostroff and Henry, as quoted by ZoBell (1946), have shown that whereas all the 15 representative aerobic bacteria of marine origin were able to utilise peptone, only 8 could utilise asparagine and only 5 could utilise di-ammonium phosphate. Burkholder (1939) showed that *Phytomonas* species, both green fluorescent and yellow pigmented, could utilise monobasic ammonium phosphate as the only source of nitrogen. On the other hand, Bhide (1948) and Patel, Dhande and Kulkarni (1951) observed

that the members of the genera *Corynebacterium* and *Xanthomonas*, formerly parts of the genus *Phytomonas*, do not utilise nitrogen from inorganic sources. McNew (1938) working with 10 strains of *Phytomonas stewarti* observed that 2 slightly virulent ones failed to utilise inorganic nitrogen, while the most virulent ones reduced nitrate to nitrite and produced curd in litmus milk. A series of experiments showed that the virulent cultures used inorganic nitrogen and slightly virulent ones regained virulence by passage through a suscept. Most of the virulent strains utilised ammonia-nitrogen more readily than nitrate nitrogen. Bhide (1948) reported that the virulent strains of *Corynebacterium michiganense* utilised a large number of organic nitrogenous compounds, especially amino acids as a source of nitrogen when dextrose was supplied, while weakly virulent srrains could utilise only aspartic and glutamic acids. On the other hand, Hildebrand (1954) observed that virulence of *Erwinia amylovora* showed no correlation with physiological behaviours and decline or loss in pathogenicity was not correlated with carbon or nitrogen utilisation.

TABLE 2

Provisional classification of 12 Xanthomonas species on the basis of their carbon utilisation.

Carbon sources	Good growth, good acid	Fair growth, fair acid	No growth to slight growth & no acid to slight acid
Dulcitol	*X. alfalfae*	*X. desmodii-gangetici*	*X. cassiae*
			X. cyamopsidis
	X. vignicola	*X. desmodii*	*X. erythrinae*
	X. stizolobiicola	*X. phaseoli-sojense*	*X. cajani*
		X. sesbaniae	
		X. tamarindi	
Glycerol	*X. alfalfae*	*X. desmodii-gangetici*	*X. cassiae*
	X. erythrinae	*X. cyamopsidis*	*X. vignicola*
	X. phaseoli-sojense	*X. desmodii*	*X. stizolobiicola*
		X. sesbaniae	*X. tamarindi*
			X. cajani
Mannitol	*X. alfalfae*	*X. desmodii-gangetici*	*X. vignicola*
	X. cassiae	*X. tamarindi*	*X. cyamopsidis*
	X. desmodii		*X. stizolobiicola*
	X. phaseoli-sojense		*X. erythrinae*
	X. sesbaniae		
	X. cajani		
Raffinose	*X. alfalfae*	*X. erythrinae*	*X. desmodii-gangetici*
	X. cassiae	*X. tamarindi*	*X. vignicola*
	X. cyamopsidis		*X. stizolobiicola*
	X. desmodii		*X. cajani*
	X. phaseoli-sojense		
	X. sesbaniae		

It was, therefore, thought worthwhile to test these 12 organisms in inorganic and organic nitrogenous compounds for differentiation, if any. Modified Richards' medium (Patel and Kulkarni, 1948) with nitrogenous compounds in liquid and solid forms was used and the growth characters recorded.

Utilisation of nitrogen from inorganic compounds :—

Nitrogen in the synthetic basal medium to the extent of 0.14 per cent showed that all the cultures utilised ammonium citrate, ammonium dihydrogen phosphate, ammonium nitrate, ammonium oxalate, ammon-ium sulphate and ammonium tartarate equally, while the absence of growth in nitrates of Ag, Ba, Ca, Cd, Cu and Zn can be ascribed to lethal, toxic or olygodynamic effect of such elements as suggested by Patel and Kulkarni (1948). Sodium nitrite has always been toxic. Differential growth was, however, observed with nitrates ofpotash, sodium and magnesium, the last being the poorest. These results are in complete conformity with those on Czapek's and synthetic nitrate media from which *Xanthomonas alfalfae*, *X. cassiae*, *X. cyamopsidis*, *X. sesbaniae* and X. *cajani* could utilise nitrogen from nitrate. It is, thus, clear that the *Xanthomonas* species, even though passed through their suscepts, are not able to utilise nitrogen from nitrates to an equal extent. It is evident that these organisms differently utilised nitrates other than ammoniacal and not that they are unable to utilise inorganic nitrogen, as reported by Patel, Dhande and Kulkarni (1951).

Utilisation of nitrogen from organic compounds :—

Each of the organic compounds to the extent of 0.1 per cent was added to the synthetic basal medium with and without dextrose and results record- ed. It was seen that the organisms utilised nitrogen from DL-a-Alanine, L-Asparagine, Aspartic acid, Creatine, Creatinine, L-Cystine, Guanidine hydrochloride, Glycine, Glucocyamine, DL-Leucine, DL-Methionine, DL-Norvaline, DL-Ornithine hydrobromide, DL-d-Phenyl alamine, DL-Serine, DL-Threonine, Tryptophane, Tyrosine, Urea and Valine but DL-Norleucine when dextrose was supplied as an additional source of carbon. Both the glutamic acid and proteose peptone served as sources of carbon and nitrogen, though addition of dextrose enhanced it. The results are in conformity with those of Patel, Dhande and Kulkarni (1951), who also showed no differences in growth with organic nitrogenous compounds.

Effect of Medium and Dye on Growth

It is well-known that crystal violet and sodium or potassium dichromate inhibit growth of the Gram-positive and Gram-negative bacteria respectively at certain concentrations only. This fact had been taken advantage of by several workers in isolating and classifying micro-organisms. Patel (1926) used crystal violet in a selective medium for isolating *Agrobacterium tumefaciens* and Wilson (1938) for isolating *Bacterium medicaginis* var. *phaseolicola*. Martin, Lowther and Leach (1943) found that *Erwinia carotovora* could be suppressed by potassium

or sodium dichromate in order to get pure colonies of *Corynebacterium sepedonicum*. Patel, Dhande and Kulkarni (1951) also used crystal violet in a synthetic nitrate medium to differentiate species of *Xanthomonas*.

As shown previously that these organisms have differential ability to utilise nitrogen from nitrate salt for growth, it was thought worthwhile to try a few more media supporting equal growth of the organisms and then ascertain the effect of different concentrations of crystal violet, malachite green and potassium dichromate on growth. Accordingly, 1 : 100 aqueous solutions of these 3 dyes were prepared and added separately so as to secure concentrations varying from 1 : 50,000 to 1 : 1,000. The media were (*i*) Modified Richards' agar, (*ii*) Peptone dextrose agar, (*iii*) Synthetic carbohydrate agar and (*iv*) Potato dextrose agar. The plates were divided into 6 sectors each and a loop of a culture from the peptone dextrose liquid was drawn in each sector. The observations made after the usual interval are recorded in Table 3. Plates without dyes were kept as controls.

It is clear from Table 3 that crystal violet has a varied effect in different media. In Modified Richards' medium due to differential growth of the organisms on nitrate, *Xanthomonas desmodii-gangetici*, *X. vignicola*, *X. stizolobiicola*, *X. erythrinae*, *X. desmodii*, *X. phaseolisojense* and *X. tamarindi* made no growth even at 1 : 50,000 while *Xanthomonas alfalfae*, *X. cassiae*, *X. cyamopsidis*, *X. sesbaniae* and *X. cajani* grew well at 1 : 50,000 to 1 : 1,000. A little inhibitory effect of the dye on these 5 organisms on nitrate medium and that on all the organisms on peptone dextrose and potato dextrose agar media was observed at 1 : 1,000 and it is felt that at higher concentrations all *Xanthomonas* species will be inhibited. All made good to excellent growth on peptone dextrose and potato dextrose agar media with crystal violet concentrations varying from 1 : 50,000 to 1 : 1,000. Thus, it seems that crystal violet has no delimiting effect on species of *Xanthomonas* but the medium plays an important role. It was interesting to note that crystal violet at 1 : 20,000 strength and above in synthetic carbohydrate medium did not support growth. However, all grew excellently in controls and fairly at 1 : 50,000. Naturally, a chemical reaction must have taken place between crystal violet and the medium. Tilley (1939) observed such toxic effect of crystal violet in the presence of di-sodium phosphate on Gram negative bacteria.

It is clear that the observations on growth in different media at 1 : 50,000 of crystal violet, 1 : 50,000 to 1 : 6666 of malachite green and 1 : 50,000 to 1 : 20,000 of potassium dichromate are the same. Malachite green and potassium dichromate at 1 : 3333 and 1 : 6666 respectively inhibit the growth completely in all the media as against their pronounced toxicity even at lower concentrations in synthetic media (MR & SC).

Thus, it is clear that these 3 dyes are not useful in delimiting the species. They may, however, help in classifying them according to their Gram reaction. How the genera of the Gram negative group, comparitively a bigger one than the Gram positive, behave differently to these dyes remains to be seen.

TABLE 3

Effect of medium and different concentrations of crystal violet, malachite green and potassium dichromate on growth of the organisms

Organisms	Crystal violet								Malachite green												Potassium dichromate											
	1 : 50,000				1 : 20,000 to 1 : 1,000				1 : 50,000 to 1 : 6,666				1 : 5,000				1 : 3,333				1 : 50,000 to 1 : 20,000				1 : 10,000				1 : 6,666			
	MR	*PD*	*SC*	*PM*	*MR*	*PD*	*SC*	*PM*	*MR*	*PD*	*SC*	*PM*	*MR*	*PD*	*SC*	*PM*	*MR*	*PD*	*SC*	*PM*	*MR*	*PD*	*SC*	*PM*	*MR*	*PD*	*SC*	*PM*	*MR*	*PD*	*SC*	*PM*
X. alfalfae	+	+	+	+	+	+	—	+	+	+	+	+	—	+	—	+	—	—	—	—	+	+	+	+	—	+	—	+	—	—	—	—
X. desmodii-gangetici	—	+	+	+	—	+	—	+	—	+	+	+	—	+	—	+	—	—	—	—	—	+	+	+	—	+	—	+	—	—	—	—
X. cassiae	+	+	+	+	+	+	—	+	+	+	+	+	—	+	—	+	—	—	—	—	+	+	+	+	—	+	—	+	—	—	—	—
X. vignicola	—	+	+	+	—	+	—	+	—	+	+	+	—	+	—	+	—	—	—	—	—	+	+	+	—	+	—	+	—	—	—	—
X. cyamopsidis	+	+	+	+	+	+	—	+	+	+	+	+	—	+	—	+	—	—	—	—	+	+	+	+	—	+	—	+	—	—	—	—
X. stizolobiicola	—	+	+	+	—	+	—	+	—	+	+	+	—	+	—	+	—	—	—	—	—	+	+	+	—	+	—	+	—	—	—	—
X. erythrinae	—	+	+	+	—	+	—	+	—	+	+	+	—	+	—	+	—	—	—	—	—	+	+	+	—	+	—	+	—	—	—	—
X. desmodii	—	+	+	+	—	+	—	+	—	+	+	+	—	+	—	+	—	—	—	—	—	+	+	+	—	+	—	+	—	—	—	—
X. phaseoli-sojense	—	+	+	+	—	+	—	+	—	+	+	+	—	+	—	+	—	—	—	—	—	+	+	+	—	+	—	+	—	—	—	—
X. sesbaniae	+	+	+	+	+	+	—	+	+	+	+	+	—	+	—	+	—	—	—	—	+	+	+	+	—	+	—	+	—	—	—	—
X. tamarindi	—	+	+	+	—	+	—	+	—	+	+	+	—	+	—	+	—	—	—	—	—	+	+	+	—	+	—	+	—	—	—	—
X. cajani	+	+	+	+	+	+	—	+	+	+	+	+	—	+	—	+	—	—	—	—	+	+	+	+	—	+	—	+	—	—	—	—

Note :—M. R. = Modified Richards' medium
P. D. = Peptone dextrose medium
S. C. = Synthetic carbohydrate medium
P. M. = Potato dextrose medium
— = No growth
\+ = Growth.

Host Range

Host range is a matter of practical importance, because plants listed as hosts are annuals or deciduous legumes and are generally found growing closely in the fields. Some bacterial plant pathogens can infect only one host species, while others infect numerous and even unrelated plants. However, studies on host range of any phytopathogenic organism can never be too extensive or complete, since all that is attempted by any worker is testing the available species of plants.

In the present study, two phases of the experiment are taken into consideration :—(*i*) validity of the phytopathogenic bacterial species on host basis, so far the only reliable and immediate entity, as will be seen later in the discussion, and (*ii*) as number of phytopathogenic bacterial species described from India are mostly on legumes, an attempt is made to infect as many species as possible to find out collateral hosts, if any, and the possible source of dissemination to field crops like *alfalfa*, soy-beans, *chavli* (*Vigna catjang*) and the field beans.

Four weeks old plants grown in sterilised soil and free of any disease were sprayed with a culture suspended, in sterile water. A separate atomiser was used for each culture. Host plants were kept in a moist chamber for 24 hours before and after inoculations and then transferred to a glasshouse bench. The host-range experiment was carried out for two monsoon seasons at temperatures varying from 25°-28°C. at which bacterial growth is the maximum and favourable for the devlopment of the disease in nature. Observations made regularly at intervals of 3 days conclusively showed that in the first phase of this experiment, *i.e.*, cross inoculations each pathogen was restricted to its own host showing the validity of creation of species on host basis. In the second phase of the experiment, hosts like *Acacia arabica* Willd., *A. catechu* Willd., *A. decurrens* Willd., *A melanoxylon* Br., *Alysicarpus bupleurifolius* DC., *A. hamosus* Edgew., *A. longifolius* W. & A., *A. monilifer* DC., *A. pubescens* Law., *A. rugosus* DC., *A teragonolobus* Edgew., *A. vaginalis* DC., *Arachis hypogaea* L., *Butea frondosa* Konig., *Caesalpinia pulcherrima* Swartz., *C. sepiaria* Roxb., *Cassia alata* L., *C. didymobotrya* Fraesen, *C. hirsuta* L., *C. siamea* Lam., *Centrosema pubescens* L., *Cicer arietinum* L., *Crotalaria anagyroides* H. B. & K., *C. juncea* L., *C. striata* DC., *Dolichos biflorus* L., *D. lablab* L., *Indigofera argenta* L., *I. arrecta* Benth., *I. glandulosa* Willd., *I. tinctoria* L. *Lathyrus odoratus* L., *L. sativus* L., *Leucaena glauca* L., *Melilotus indica* All., *Moringa pertygosperma* Gaertn., *Phaseolus aconitifolius* Jacq., *P. angularis* Wright, *P. coccineus* Lam., *P. lunatus* L. *P. mungo* var. *radiatus* L., *P. vulgaris* L., *Pisum arvense* L., *P. sativum* L., *Poinciana regia* Bojer, *Pueraria phaseoloides* Borth., *Sesbania aculeata* Poir., *Tephrosia candida* DC., *T. purpurea* Pers., *Trifolium alexandricum* L , *Trigonella foenum-graecum* L. and *Vigna sinensis* Endl. were tried when *Xanthomonas alfalfae* infected *M. indica*, *P. sativum*, and *T. foenum-graecum* ; *X. cassiae* infected *P. sativum* ; *X. vignicola* infected *P vulgaris* and *V. sinensis* ; *X. phaseolisojense* infected *D. biflorus*, *P. lunatus* and *P. vulgaris* and *X. tamarindi* infected *C. sepiaria* besides their respective suscepts. Kulkarni, Patel and Dhande (1952) have shown that, although *X. alfalfae* and *X. cassiae*

have a common host in *P. sativum*, the pathogen when reisolated from *P. sativum* was found restricted to the original suscept. Similarly, though *X. vignicola* and *X. phaseoli-sojense* have a common host in *P. vulgaris*, the pathogens do not cross-inoculate.

Discussion

An attempt is made in Tables 4 and 5 to record pertinent information on morphological, cultural and biochemical behaviours of 12 *Xanthomonas* species causing leaf-spots and stem canker on some species of Leguminosae. In Table 4 are shown similarities amongst themselves while Table 5 records differences.

Most of the data presented here corroborate those of Dowson (1949) and Patel, Dhande and Kulkarni (1951), who observed that all members of *Xanthomonas* were very much alike in their morphological, cultural and biochemical characters and could only be differentiated from one another on the basis of pathogenicity.

During the last 25 years, many workers, like Burkholder (1930), Clara (1934), Waldee (1945), Bhide (1948), Patel, Dhande and Kulkarni (1951) etc., studying a group like bacterial diseases of beans, *Pseudomonas*, soft-rot-producing bacteria, wilt-producing bacteria, *Xanthomonas* species and so on respectively attempted to delimit the species in a group on the basis of morphological, cultural, biochemical and serological differences, and recommended a provisional key, but such keys become less reliable as the number of organisms in a group increases. Every one showed the validity of a species on the basis of host specificity. In this respect the genus *Xanthomonas* is unique and still there are species like *X. campestris*, *X. citri*, *X. phaseoli*, etc., which attack more than one host of the related genera. On the other hand, there are more cases where only one species in a genus is affected. Attempts have also been made to delimit species in a group on the basis of their serological responses, but no correlation could be established between serological reaction and host specificity. Burkholder (1937) felt that too much emphasis on serological reactions among the plant pathogens should not be placed.

Many a time it is argued that the character of pathogenicity is not reliable as some cultures lose virulence in artificial media on subsequent transfers. Ark (1937) and Hildebrand (1954) observed variability in pathogencity of the fire blight organism. Such is not a case in this laboratory, although cultures transferred regularly on a suitable medium at regular intervals for 5-6 years have retained their pathogenecity. According to Brierley (1926), most of the bacteria are remarkably stable and this implies an extremely delicate and constant hereditary mechanism, quite comparable in its behaviouristic fixity and nicety to that in higher animals. This will remain true so long as there is no radical change in nature. Because of their similar characters, the bacterial phytopathogens, members of the colon-typhoid group and some of the soil saprophytes are considered to have a common ancestry. So far, however, there is no authentic proof of such a saprophyte grown in association with any host becoming phytopathogenic. On the other hand, such saprophytes

and phytopathogens live in nature in association for a long time and still only one or a few plants are found diseased. Thus, the phytopathogenic bacteria, even though allied to colon-typhoid group or other soil saprophytes, may be considered to have developed during the course of evolution, the character of pathogenicity demands special attention of the taxonomists. It is well pointed out by Patel and Kulkarni (1951) that unless the source of an organism is known, it is difficult to assign it a proper place in the classification. An organism with its detailed morphological, cultural and biochemical responses, but without information on its source, will take years to assign it an exact place in classification. The creation of a new family Phytobacteriaceae by Patel and Kulkarni (1951), therefore, to accommodate the phytopathogenic bacteria demands serious consideration.

Report of new bacterial diseases does not necessarily mean creation of new species. The endeavour should be to inoculate the available related and unrelated hosts found in the vicinity. Cross inoculations should be made, if a common host is found for two or more organisms. After this much, care, creation of a new species be advocated if found host specific or different in morphological, cultural and biochemical responses.

This comparative study was undertaken with a view to throw some light on the nutritional aspects of these 12 *Xanthomonas* species exclusively parasitising Leguminosae. Their differential growth in synthetic nitrate and Czapek's media as well as in tri-saccharides and alcohols is an elucidation to some of the salient characters of the group in general and such differences may be present in other *Xanthomonas* species parasitising non-leguminous plants also.

Perhaps, this is the only laboratory from which 38 phytopathogenic bacteria have been reported. Of these, 34 belong to the genus *Xanthomonas*. Elliott (1951) records about 195 phytopathogenic bacteria, of which 4 belong to *Agrobacterium*, 5 to *Bacterium*, 10 to *Corynebacterium*, 22 to *Erwinia*, 92 to *Pseudomonas* and 62 to *Xanthomonas*. Even though the climatic conditions under which these 34 *Xanthomonas* species flourish are much different, their characters are practically the same in Asia, Europe, the U.S.A. etc. Question then arises as to why from India, an Asian country, a larger number of *Xanthomonas* species are reported than from elsewhere? Why are there only a few *Pseudomonas* species (*Phytobacterium*) only? Perhaps, because the bacterial plant diseases are reported from a few square miles round about Poona, a very negligible area, as against the vast unexplored area of India which includes thick forests of Gir, Dangs, Mount Abu, Tarai, Assam, Nilgiris, etc. Excursion, to such areas and detailed investigations of bacterial plant diseases from them might throw some light on these questions if, however, climatic conditions do not delimit a particular genus to disseminate or propagate.

Lastly, no possible explanation can be forwarded as to why out of 34 *Xanthomonas* species reported from this laboratory, 50 per cent parasitise Leguminosae only?

TABLE 4

Comparison of the morphological, cultural and biochemical responses of 12 Xanthomonas species.

Characters	*X. alfalfae* *X. desmodii-gangetici* *X. cassiae* *X. vignicola* *X. cyamopsidis* *X. stizolobiicola* *X. erythrinae* *X. desmodii* *X. phaseoli-sojense* *X. sesbaniae* *X. tamarindi* *X. cajani*
Gram stain	Negative
Motility	Motile
Gelatin	Liquefied
Starch	Hydrolysed
Casein	Digested
Litmus milk	Reduced with acid reaction
Sodium chloride	Upto 3 per cent
Indol	Not produced
Hydrogen sulphide	Produced
Ammonia	Produced from peptone
Nitrite	Not produced
Mono-saccharides	Acid produced
Di-saccharides	—do—
Poly-saccharides	—do—
Glucosides	Not utilised
Acetic, Citric & Lactic acids	Alkali produced
Inorganic ammoniacal nitrogen	Utilised in presence of dextrose
Organic nitrogenous compounds	—do—
Glutamic acid & Proteose peptone	Utilised in absence of dextrose
Norleucine	Not utilised
Crystal violet	All grow at 1:1000
Malachite green	All inhibited at 1:3333
Potassium dichromate	All inhibited at 1:6666

TABLE 5

(a) Differences in cultural and biochemical responses of 12 Xanthomonas species

Character	X. alfalfae	X. desmodii-gangetici	X. cassiae	X. vignicola	X. cyamopsidis	X. stizolobiicola	X. erythrinae	X. desmodii	X. phaseoli-soiense	X. sesbaniae	X. tamarindi	X. cajani
Czapek's	++	+	++	+	+	+	+	+	+	++	+	+
Loeffler's	++	+	++	+	+	+	+	+	+	++	+	+
Dulcitol	+	+	+	+	+	+	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+

Note : + + + =Excellent growth, + + =good growth, + =fair growth and − =no growth

TABLE 5 (*Continued*)

(*b*) *Artificial temporary key to classify 12 Xanthomonas species on the basis of cultural and biochemical responses.*

I. Nitrogen from potassium and sodium nitrate utilised	
A. Loeffler's slightly liquefied	
(*i*) Dulcitol, glycerol, mannitol and raffinose utilised	*X. alfalfae*
(*ii*) Only raffinose utilised	*X. cyamopsidis*
B. Loeffler's fairly liquefied	
(*i*) Mannitol and raffinose strongly, but dulcitol and glycerol poorly utilised	*X. cassiae*
(*ii*) Mannitol and raffinose strongly, but dulcitol and glaycerol fairly utilised	*X. sesbaniae*
(*iii*) Only mannitol strongly utilised	*X. cajani*
II. Nitrogen from potassium and sodium nitrate not utilised	
A. Loeffler's slightly liquefied	*X. phaseoli-sojense*
B. Loeffler's fairly liquefied	
(*i*) Only glycerol strongly utilised	*X. erythrinae*
(*ii*) Only mannitol strongly utilised	*X. desmodii*
(*iii*) Dulcitol, glycerol and mannitol fairly utilised	*X. desmodii-gangetici*
(*iv*) Raffinose fairly utilised	*X. tamarindi*
C. Loeffler's strongly liquefied	*X. vignicola* and *X. stizolobiicola*

It will be seen from Table 4 that the organisms are hardly distinguishable by their morphological, cultural and biochemical behaviours. Table 5 shows some differential characters of each organism on the basis of which they are distinguished from one another. However, *Xanthomonas vignicola* and *X. stizolobiicola* could not be separated from each other and it may be that with an increase in the number of organisms in a group such keys may be more confusing. As suggested by Wernham (1948) and Patel, Dhande and Kulkarni (1951), host specificity be considered the only reliable character for establishing a new species.

Summary

12 *Xanthomonas* species inciting leaf-spots and stem canker in Leguminosae were studied for their morphological, cultural, biochemical and host responses.

All species were rod-shaped, Gram negative, motile by single polar flagellum and capsulated. All grew well in potato dextrose and nutrient dextrose agars; liquefied gelatine; hydrolysed starch; digested casein; produced hydrogen sulphide and ammonia from peptone; indol and nitrate not produced; formed acid in mono-, di-, poly-saccharides; alkali in acetic, citric and lactic acids; no growth in glucosides; utilised all ammonical compounds, but not nitrates of heavy metals; amino acids utilised in presence of dextrose; glutamic acid and proteose peptone supplied both the nitrogen and carbon; tolerated sodium chloride upto 3 per cent; inhibited slightly at 1 : 1,000 crystal violet, completely at 1 : 3,333 malachite green and 1 : 6,666 potassium dichromate.

Only *Xanthomonas alfalfae, X. cassiae, X. cyamopsidis, X. sesbaniae* and *X. cajani* utilised nitrogen from nitrates of potassium and sodium but did not produce ammonia or nitrite. Differential growth was exhibited by all in dulcitol, glycerol, mannitol and raffinose and on Loeffler's blood serum.

The common host for *X. alfalfae* and *X. cassiae* is *Pisum sativum* and that for *X. vignicola* and *X. phaseoli-sojens* is *Phaseolus vulgaris. X. alfalfae* infects *Melilotus indica* and *Trigonella foenum-graecum; X. vignicola* infects *Vigna sinensis; X. phaseoli-sojense* infects *Dolichos biflorus* and *Phaseolus lunatus* and X. *tamarindi* infects *Caesalpinia sepiaria* in addition to their own specific hosts.

Accommodation of phytopathogenic bacteria, otherwise undifferentiated from colon-typhold group and some of the soil saprophytes, into the new family Phytobacteriaceae seems to be great relief for their immediate identity and in a genus like *Xanthomonas*, host specificity is the only reliable and immediate character to establish a new species.

Acknowledgements

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REFERENCES

Ark, P. A. (1937) Variability of the fire blight organism, *Erwinia amylovora*. Phytopathology, **27**: 1-28.

Bergey, D. H. *et al.* (1948) Manual of determinative bacteriology. Bailliere, Tindall & Cox., London W. C. 2.

Berridge, E. M. (1924) The influence of hydrogen-ion concentration on the growth of certain bacterial plant parasites and saprophytes. Ann. appl. Biol., **11**: 73-85.

Bhide, V. P. (1948) A comparative study of some wilt producing phytopathogenic bacteria. Indian Phytopath., **1**: 70-91.

——————— (1949) Stomatal invasion of cabbage by *Xanthomonas campestris* (Pammel) Dowson. Indian Phytopath., **2**: 2-3.

Brierley, W. B. (1926) Variation in fungi and bacteria. Proc. Intern. Congr. Plant Sci., Ithaca, N. Y., **2**: 1629-1654 (original not seen).

Burkholder, W.H. (1930) The bacterial diseases of the beans: a comparative study. Mem. New York (Cornell) agric. Expt. Stat., 127.

——————— (1932) Carbohydrate fermentation by certain closely related species in the genus *Phytomonas*. Phytopathology, **22**: 699-707.

——————— (1937) A bacterial leaf-spot of *Geranium*. Phytopathology, **27**: 554-560.

——————— (1937) Serological reactions for the determination of bacterial plant pathogens. Phytopathology, **27**: 572-574.

——————— (1939) The taxonomy and nomenclature of the phytopathogenic bacteria. Phytopathology, **29**: 128-136.

———————and M. P. Starr (1948) The generic and specific characters of phytopathogenic species of *Pseudomonas* and *Xanthomonas*. Phytopathology, **38**: 444-502.

Clara, F. M. (1934) A comparative study of the green fluorescent bacterial plant pathogens. Mem. Cornell agric. Expt. Stat., 159.

Conn, H. J. and R. S. Breed (1919) The use of the nitrate reduction test in characterising bacteria. J. Bact., **3**: 267-290.

Dowson, W. J.	(1949)	Manual of bacterial plant diseases. Adam and Charles Black, London W. 1.
Elliot, Charlotte	(1951)	Manual of bacterial plant pathogens. Waltham, Mass., U.S.A.
Hildebrand, E. M.	(1954)	Relative stability of fire blight bacteria. Phytopathology, **44** : 192-197.
Kent, G. C.	(1942)	Nitrogen sources utilised by some bacterial plant pathogens. Phytopathology, **32** : 12 (abstract).
Kulkarni, Y. S., M. K. Patel and G. W. Dhande	(1952)	Bacterial leaf-spot of *Cassia tora* L. Indian Phytopath. **5** : 76-88.
Levine, Max	(1933)	An introduction to laboratory technique in bacteriology. MacMillan Company, New York.
Martin, E. A., C. V. Lowther and J. G. Leach	(1943)	A differential medium for the isolation of *Phytomonas sepedonicum*. Phytopathology, **33** : 406-407.
McNew, G. L.	(1938)	The relation of nitrogen nutrition to virulence in *Phytomonas stewarti*. Phytopathology, **28** : 769-787.
Patel, M. K.	(1926)	An improved method of isolating *Pseudomonas tumefaciens*. Phytopathology, **16** : 577.
————and Y. S. Kulkarni	(1949)	Nitrogen utilisation by *Xanthomonas malvacearum* (Sm.) Dowson. Indian Phytopath., **2** : 62-64.
——, N. B. Kulkarni and S. R. Gaekwad	(1950)	Staining bacterial flagella. Curr. Sci., **19** : 322-323.
————and Y. S. Kulkarni	(1951)	Nomenclature of bacterial plant pathogens. Indian Phytopath., **4** : 74-84.
——, G.W. Dhande and Y.S. Kulkarni	(1951)	Studies in some species of *Xanthomonas*. Indian Phytopath., **4** : 123-140.
——, V.V. Bhatt and Y. S. Kulkarni	(1952)	Some phytopathogenic bacteria requiring reinvestigation. Indian Phytopath., **5** : 116-120.
Riker, A. J., F. R. Jones and C. Davis	(1937)	Bacterial leaf-spot of alfalfa. J. agric. Res., **51** : 177-182.
Society of American Bacteriologists	(1945)	Manual of Methods for pure culture study of bacteria. Geneva, New York.
Tilley, F. W.	(1939)	Bacterial efficiency of certain aniline dyes. J. agric. Res., **58** : 941-946.

Vaughan, E. K. (1943) Forms and concentrations of nitrogen utilised by *Phytomonas solanacearum*. Phytopathology, **33** : 14-15 (abstract).

Vaughan, Reese and Max, Levine (1936) Hydrogen sulphide production as a differential test in the colon group. J. Bact., **32** : 65-73.

Waldee, E. L. (1945) Comparative studies of some peritrichous bacteria. Iowa State College J. Sci., **19** : 435-484.

Wernham, C. C. (1948) The species value of pathogenicity in the genus *Xanthomonas*. Phytopathology, **38** : 283-291.

Wilson, R. D. (1938) A selective medium for *Bacterium medicaginis* var. *phaseolicola* (Burkholder) Link and Hull. J. Austr. Inst. agric. Sci., **4** : 47-49.

Wolf, F. A. and I. V. Shunk (1921) Tolerance to acids of certain bacterial plant pathogens. Phytopathology, **11** : 244-250.

ZoBell, C. E. (1946) Marine Microbiology. Waltham. Mass., U.S.A.

PHYTOPATHOLOGICAL NOTES

Control of "damping off" in tobacco seedbeds in Bombay State—V. P. Bhide and M. K. Patel. "Damping-off" in tobacco seedbeds, primarily caused by *Pythium deBaryanum* Hesse, is a serious disease in Kaira District of Bombay State and causes heavy reductions in the stand of seedlings. In years when the disease is severe, enough seedlings are not available for transplanting the area reserved for tobacco, resulting in heavy losses to the tobacco grower.

Tobacco seedlings are usually raised in beds 16′×4′ and sowing is done late in June or early in July after the monsoon has well established. "Damping-off" is very serious in July-August, during the breaks in monsoon, when soil temperature and moisture are optimum for the development of the pathogen. Both pre-and post-emergence "damping off" are common but the disease is usually very severe in seedlings 15-20 days old. Affected seedlings fall to the ground and wilt forming large patches of dead seedlings in the seedbed. The disease stops late in September or early in October. *Pythium deBaryanum* is isolated from diseased seedlings in majority of cases, though other Pythia and species of Fusarium are not uncommonly associated with diseased seedlings.

Experiments were started in 1940-41 on the Tobocco Breeding Station, Nadiad (Dist. Kaira) with a view to evolving a method for control of "damping-off" through soil sterilisation with fungicidal sprays and were continued for four years. During this period, the following fungicides were tested in different concentrations:

Perenox (a proprietory copper fungicide manufactured by the Imperial Chemical Industries Ltd., England).....0.5 and 0.25%

Mycol (a proprietory copper fungicide sold by W. J. Craven and Co., Evesham, England). 0.25 and 0.125%

Bordeaux Mixture .....2-2-50 and 3-3-50.

Untreated beds served as control. Each bed (4′×4′) was enclosed in a galvanised iron sheet frame nine inches high, provided with holes on all sides to drain off excess water and buried to a depth of one inch. The soil was artificially infested every year (except in 1940-41) with the "damping-off" fungus by mixing with it fungus grown on carrot decoction agar; five days' growth on 100 cc. of the medium was used for every one square foot of seed bed area. Four gms. of tobacco seed (variety K. 49) were uniformly sown in each bed and watering was done whenever necessary. The fungicides were applied to the beds at weekly intervals starting a week before sowing was done and the treatments were stopped when the disease had ceased in untreated beds, which was usually in September. About six applications of fungicides were necessary.

Observations on the number of seedlings damped-off and the area damped-off (in sq.cm.) were taken every two days; a glass plate marked off in sq. cm. was used for measuring the areas damped-off.

The results showed that Perenox in a concentration of 0.5 per cent consistently gave good control of damping-off; bordeaux mixture 2-2-50 was next best but there was no difference between the two strengths of Bordeaux mixture tested.

The authors are indebted to Dr. B. N. Uppal, formerly Plant Pathologist to Government, B. S., Poona, for advice and guidance during this investigation. Plant Pathological Laboratory, College of Agriculture, Poona.

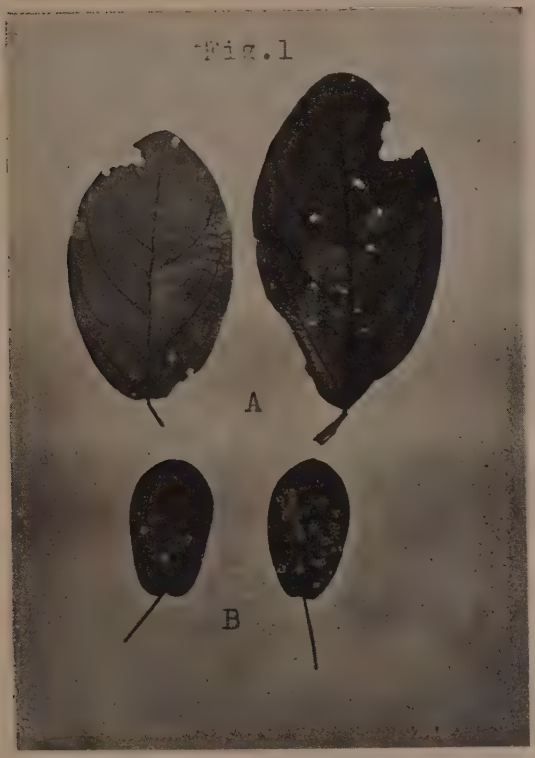
A New Variety of Xanthomonas alysicarpi—M. K. Patel, V. V. Bhatt and G. W. Dhande. Recently Bhatt and Patel (1954) described *Xanthomonas alysicarpi* on *Alysicarpus rugosus* DC. producing leaf-spots and blight at Jalgaon. On excursions to Parvati Hills of Poona, a bacterial disease was observed on *Alysicarpus vaginalis* DC. In the beginning, *A. vaginalis* was thought to be a collateral host of *X. alysicarpi*. However, on detailed study, the pathogen on *A. vaginalis* was found different from *X. alysicarpi*.

The pathogen produces a few, round, water-soaked areas which on further development of the disease turn deep brown and produce a small yellow halo around measuring 0.6—1.3 mm. When numerous spots coalesce, the whole leaf becomes yellow and dries up. However, blight symptom from a few spots is never observed in *A. vaginalis* as observed in *A. rugosus* (Fig. 1.). The morphological, cultural and biochemical characters of the pathogen in question are as follows :—

Short rods, single polar flagellum; $0.5 \times 1.7\mu$ in size; Gram negative; capsulated; non-spore former; on potato dextrose agar plates, colonies are circular, with entire margin, smooth, shining, raised, measuring 1.8 cm. after 8 days, colour Pinard yellow; gelatin liquefied; starch hydrolysed; casein digested; milk peptonised and litmus reduced with slight acid reaction; hydrogen sulphide and ammonia produced from peptone; nitrite not produced; no growth in synthetic nitrate and Czapek's media; acid without gas from arabinose, dextrose, lactose and starch; no growth in salicin; optimum temperature for growth 27°-30°C.; thermal death point about 51°C.

From the above characters it is beyond doubt that the pathogen belongs to the genus *Xanthomonas* and does not differ much from *X. alysicarpi*. However, on host range studies it was found that the pathogen infects only *Alysicarpus vaginalis* but not *A. bupleurifolius, A. hamosus, A. longifolius, A. monilifer, A. pubescens, A. rugosus, A. tetragonolobus,* related and unrelated hosts reported by Patel and Kulkarni (1953) and others on which bacterial diseases are reported from this laboratory.

As the members of the genus *Xanthomonas* are unique in having many similar characters but alloted a *novum* status on their host reaction in which they are highly specific, similarly the pathogen even though similar to *X. alysicarpi* in many of the morphological, cultural and biochemical behaviours but being highly specific to its host, has been raised to a status of novum variety *viz. Xanthomonas alysicarpi* var.



A. Blight in *A. rugosus* B. Leaf-spots in *A. vaginalis*

vaginalidis. This is the second instance in this laboratory where so much of host specialisation of a pathogen is found on species of a single plant genus, the first instance being *X. desmodii* and *X. desmodii* var. *gangetici* on *Desmodium diffusum* and *Desmodium gangeticum* respectively.

Xanthomonas alysicarpi var. *vaginalidis* Patel, Bhatt and Dhande nov. var. on leaves of *Alysicarpus vaginalis* DC. Found at Parvati Hills, Poona, in June, 1954. Leg. V. V. Bhatt—Plant pathological Lab., College of Agriculture, Poona 5, 22nd Oct., 1954.

References

Bhatt, V. V. and M. K. Patel (1954) *Curr. Sci.* **23** : 165

Patel, M. K. and Y. S. Kulkarni (1953) *Indian Phytopathology* **6** : 131-140

INDIAN PHYTOPATHOLOGICAL SOCIETY, DELHI.

Receipts and payments account for the year ended 31st December 1953.

RECEIPTS

	Rs.	A.	P.	Rs.	A.	P.
To Balances on 1st January 1953 :						
Cash in hand ...	122	3	3			
Balance with Lloyds Bank Ltd., New Delhi, in Savings Account ...	5,129	5	6	5,251	8	9
,, Admission Fees ...				100	0	0
,, Life Membership Fees				150	0	0
,, *Subscription :*						
For 1951 ...	40	0	0			
,, 1952 ...	270	0	0			
,, 1953 ...	463	5	0			
,, 1954 ...	10	0	0			
,, 1955 ...	10	0	0	793	5	0
,, Journal Subscriptions ...				2,353	0	2
,, Reprints ...				75	5	0
,, Advertisements in Journal ...				166	0	0
,, Interest on Savings Account ...				72	13	0
,, Publication Grants ...				500	0	0
Total ...				9,461	15	11

PAYMENTS

	Rs.	A.	P.	Rs.	A.	P.
By Establishment ...				340	0	0
,, Postage ...				111	7	0
,, Printing & Stationery				2,176	7	9
,, Travelling & Conveyance ...				140	11	0
,, Bank Charges ...				16	1	0
,, Audit Fees ...				25	0	0
,, Miscellaneous Expenses				7	4	0
,, *Balances on 31st December 1953 :*						
Cash on hand ...	51	3	6			
Balance with Lloyds Bank Ltd., New Delhi, in Savings Account ...	6,593	13	8	6,645	1	2
Total ...				9,461	15	11

We have examined the annexed Receipts and Payments Account for the year ended 31st December 1953, and have to report as under:

(*a*) In respect of the twelve years Post Office National Savings Certificates for Rs. 5,000/- we have been furnished with a certificate from Lloyds Bank Ltd., New Delhi, in support of the Safe Custody with them of the securities as on 31st December 1953. We have not personally verified the certificates.

(*b*) The attention of the members is invited to the resolution contained in para 7 of the proceedings in members meeting of 3rd January 1950. The resolution was to the effect that whenever the funds of the Society exceeded Rs. 3,500/-, such excess should be invested in National Cash Savings Certificates. The balance in Lloyds Bank Limited since the 3rd January 1950 has been in excess of Rs. 3,500/- and it was Rs. 6,593/13/8 on 31st December 1953. It would appear that this resolution dated 3rd January 1950 has not been put into effect.

Subject to these remarks, we certify that the Receipts and Payments Account of your Society as on 31st December 1953 has been found correct in accordance with the books and information supplied.

New Delhi,
Dated: 3rd May, 1955.

R. PRASADA
Secretary-Treasurer

AIYAR & Co.
Chartered Accountants.

INDIAN PHYTOPATHOLOGICAL SOCIETY, DELHI.

Receipts and payments account for the year ended 31st December 1954.

RECEIPTS

	Rs.	A.	P.	Rs.	A.	P.
To Balances on 1st January 1954 :						
Cash on hand ...	51	3	6			
Balance with Lloyds Bank Ltd., New Delhi, in Savings Account ...	6,593	13	8	6,645	1	2
,, Admission Fees ...				80	0	0
,, Life Membership Fees				430	0	0
Subscription :						
1952 ...	40	0	0			
1953 ...	210	6	0			
1954 ...	418	8	0			
1955 ...	9	3	0	678	1	0
,, Journal Subscription ...				1,535	1	6
,, Reprints ...				622	5	0
,, Advertisements in Journal ...				144	0	0
,, Interest on Savings Account ...				85	0	0
,, Publication Grants ...				500	0	0
Total				10,719	8	8

PAYMENTS

	Rs.	A.	P.	Rs.	A.	P.
By Establishment ...				300	0	0
,, Postage ...				299	7	3
,, Printing & Stationery...				4,902	5	3
,, Travelling & Conveyance ...				27	8	0
,, Bank charges ...				9	6	0
,, *Balances on 31st December 1954 :*						
Cash on hand ...	136	12	3			
Balance with Lloyds Bank Ltd., New Delhi, in Savings Account ...	5,044	1	11	5,180	14	2
Total ...				10,719	8	8

We have examined the annexed Receipts and Payments Account for the year ended 31st December 1954, and have to report as follows :

(*a*) In respect of the twelve years Post Office National Savings Certificates for Rs. 5,000/- we have been furnished with a certificate from Lloyds Bank Ltd., New Delhi, in support of the Safe Custody with them of the securities as on 31st December 1954. We have not personally verified the certificates.

(*b*) We refer to para 2 of our report of date on the Society's Accounts for the year ended 31st December 1953. The position continues to be the same. The balance with Lloyds Bank Ltd., New Delhi, on 31st December 1954 stood at Rs. 5,044/1/11.

(*c*) Under head "Printing & Stationery", a sum of Rs. 4,902/5/3 was spent during the year as against Rs. 2,176/7/9 in 1953. The increase is explained by the fact that in 1954 payments were made towards three numbers (Vol. V No. 2, Vol. VI Nos. 1 and 2) of the journal "Indian Phytopathology" as against for two numbers in 1953. More reprints of articles from the journal were printed during the year (the cost of which amounted to Rs. 1,147/10/6 against Rs. 83/14/6 in 1953), which also partly accounts for the increase in the expenditure under this head.

Subject to these remarks, we certify that the Receipts and Payment Account of your Society as on 31st December 1954 has been found correct in accordance with the books and information supplied.

New Delhi,
Dated : 3rd May, 1955

R. PRASADA
Secretary-Treasurer

AIYAR & Co.
Chartered Accountants.

EIGHTH ANNUAL REPORT OF THE INDIAN PHYTOPATHOLOGICAL SOCIETY (1954)

I am presenting herewith the Eighth Annual Report of the Indian Phytopathological Society for the year 1954. During this year eight new members were admitted to the Society bringing the total number to 188. The total number of subscribers at present is 126.

Three numbers of Indian Phytopathology were published during the year. Now that more papers are being received from the members for publication in our Jourual, it should be possible to issue three numbers in 1955, thus bringing the publication up-to-date.

It is a matter of great satisfaction to report that the financial position of the Society continues to be sound, inspite of the fact that, apart from a sum of Rs. 500/- received as publication grant from the National Institute of Sciences of India to whom our thanks are due, no other monetary aid was received from any other body. The year opened with a balance of Rs. 6,645/1/2, in addition to a sum of Rs. 5,000/- invested in National Savings Certificates which are kept in the custody of Lloyd's Bank Ltd., New Delhi. Receipts during the year amounted to Rs. 4,074-7-6 and Payments amounted to Rs. 5,538-10-6. The increase in expenditure is due to the fact that in 1954 payments were made towards three numbers (Vol. V, No. 2, and Vol. VI Nos. 1 and 2) as against two normally paid for every year. Besides, several Membership fees and subscriptions for 1954 are due which, it is expected, would be paid during the course of 1955.

Duly audited Statements of Receipts and Payments for 1953 and 1954 along with the audit Reports are placed before you for your consideration.

I take this opportunity to express my grateful thanks to the members of the Society, the President and the Councillors for their kind support. I am also grateful to Dr. R. S. Vasudeva, Head of the Division of Mycology, I.A.RI., for providing necessary facilities and to Dr. S. P, Raychaudhuri for helping me in various ways. Thanks are also due to Messrs. Aiyar & Co., Chartered Accountants, for auditing the accounts of the Society.

R. PRASADA
Secretary-Treasurer.

ANNOUNCEMENTS

For certain reasons the Annual General Meeting of the Indian Phytopathological Society, which used to be held every year during the Science Congress week in the past, could not be convened this year (1955). Under the circumstances, the ballot papers for election to the Council for 1955 were opened in Delhi in the presence of Dr. P. R. Mehta, the retiring Vice-President, Dr. M. R. S. Iyengar, the retiring Councillor of the Northern Zone, and myself. After the counting of the votes the following were declared to have been elected to the Council of the Society for 1955 :—

President :	Dr. K. D. Bagchee, Dehra Dun
Vice-President :	Dr. M. J. Thirumalachar, Poona
Councillors :	Dr. M. R. S. Iyengar, New Delhi
	Dr. S. Sinha, Agra
	Dr. S. Chowdhury, Jorhat (Assam)
	Dr. S. Vaheeduddin, Hyderabad. Dn.
	Dr. M. K. Patel, Poona
	Dr. T, S. Sadasivan, Madras

(Dr. R. Prasada, New Delhi, continues to hold the office of the Secretary-Treasurer to which he was elected for three years in 1953).

R. PRASADA
Secretary-Treasurer
Indian Phytopathological Society
Pusa Building, New Delhi-12.

NATIONAL REGISTER OF SCIENTIFIC & TECHNICAL PERSONNEL

A letter received from CSIR in connection with the compilation of the National Register of Scientific & Technical Personnel in India is reproduced below for the information of those members who have not already sent in their details on the questionnaire form. They are advised to obtain the forms direct from the Secretary, CSIR, Old Mill Road, New Delhi.

Dear Sir,

You are aware that in pursuance of the recommendation of the Scientific Manpower Committee, the Council of Scientific & Industrial Research has been engaged in the compilation of the National Register of Scientific & Technical Personnel in India. The object of this undertaking is to assess the existing scientific manpower in the context of the requirements of the country.

On the basis of data collected earlier with your co-operation, three volumes in several parts have already been published; Volume I (Parts I, II & III) comprising of Engineers, Volume II (Parts I, II & III) of Medical Personnel and Volume III (Parts I & II) of Scientists & Technologists. It is now proposed to publish Part III of the last volume to include such scientists & technologists as have not furnished the required information so far. This part also alike the previous two parts, which include about 10,000 names of qualified scientists & technologists, will cover the following categories :—

1. Chemists & Chemical Technologists.
2. Pharmacists.
3. Physicists & Applied Physicists.
4. Mathematicians & Statisticians,
5. Botanists.
6. Zoologists.
7. Biologists.
8. Meteorologists.
9. Geologists.
10. Metallurgists.
11. Agricultural Scientists.

I, therefore, request you to send the latest list of members of your society in order to enable this office to contact such persons as have not yet been registered. I am also enclosing a few copies of questionnaire forms which may please be circulated to the new members of your society and more copies, if necessary may please be typed or obtained from this office.

Your co-operation is also requested in giving this publicity through the publications of your Society.

Thanking you for your co-operation.

Yours faithfully,
(P. Narain)
Officer-in-Charge.

Prof : M. Sayeeduddin
Head, Dept : of Botany,
Principal and Dean.

Department of Botany
UNIVERSITY COLLEGE OF SCIENCE
OSMANIA UNIVERSITY,
Hyderabad-Deccan-7.
Dated 16-5-55.

Dear Dr. Prasad,

As you know the 43rd Session of the Indian Science Congress Association will be held at Agra in January, 1956. I hope you and some of your colleagues will attend the session and contribute your share by sending papers and taking part in the discussions on (1) Stain Technique (2) Modern Trends in Plant Taxonomy, towards making the activities of our section a success.

I shall feel obliged if you kindly circulate this amongst your colleagues, and request them on my behalf to contribute papers and to take part in the discussions.

The names of those members taking part in the discussions have to reach me by the end of this month. The full papars accompanied by three copies of Abstracts are to be sent to the General Secretary, Indian Science Congress Association, 1 Park Street, Calcutta-16, so as to reach him before the 15th September 1955.

Thanking you,

Yours sincerely,
President
Botany Section,
Indian Science Congress Association

INDIAN PHYTOPATHOLOGICAL SOCIETY

Instructions to Authors

Membership in the INDIAN PHYTOPATHOLOGICAL SOCIETY is prerequisite to publishing in INDIAN PHYTOPATHOLOGY but the Editorial Board may relax this rule in the case of contributions of exceptional merit and communicated with a special recommendation by a member. The Editorial Board may invite distinguished scientists to contribute articles of interest to the Society.

Contributions should be on one side of the page, double spaced, with a 1-1/4th inch margin on the left. In form and style, such as punctuation, spelling and use of italics, the manuscript should conform to the best Journals in the U.K. and U.S.A. Authors should strive for a clear and concise style of writing. The name and address of the Institution at which the work was done should be cited immediately after the SUMMARY at the end of the article on left hand side. Tables should be numbered and each table should have a heading stating briefly its contents. References to literature should be made as foot notes *only* when four or fewer citations are given. If there are more, they should be listed under 'REFERENCES' at the end of the paper and referred to by date in brackets in the body of the article. Citation should give the name of the author (or others), his (or their) initials year of publication and then the full title correctly, followed by the name of the Journals, number of the volume, a colon and page numbers. If the title is in a foreign language, then diacretic signs and capitalization should be precisely as in the original. The names of the Journal should be as abbreviated in the WORLD LIST OF PERIODICALS, 2nd Ed., 1934, but as that book may not be available to all, contributors are requested to give the titles in full. Abbreviating will in that case, be done by the Editors. If an article has not been seen in original, then that fact should be clearly stated. An example citing is given below :—

Conovor. R.A. (1948).....Studies of two viruses causing mosaic diseases of soybean. *Phytopathology*, **38** : 724-735.

Because of high cost of half-tone blocks carefully made line drawing on Bristol board in black ink will be preferred. Photographs when necessary should be printed on glossy contrast paper and be of best quality. Full page figures and photographs should be made to reduce $4 \times 6\frac{1}{2}$ inches, the standard size for all plates. Each author is allowed one page of half-tone illustration for each article or its equivalent, and the cost of half-tone blocks and paper in excess will be charged to author. Drawings must be drawn to standard scales, so that they can be compared with one another. *e.g.*, $\times 10$, $\times 50$, $\times 100$, $\times 250$, $\times 500$ etc. It is not always possible to get a magnification at a round figure with a camera lucida but the printer can readily reduce drawings at any magnification to the standard, provided a scale is added to the drawing. The scale should measure from 5 to 10 cm. the longer the better and the printer should be instructed to reduce this line to the desired magnification.

Authors are invited to consult Bisby's 'An Introduction to Taxonomy and Nomenclature of Fungi' (1945), pp. 38-41 and Riker's 'The preparation of manuscripts for *Phytopathology*, *Phytopathology* **36** : 953-977, 1946, before preparing their mss. and figures.

Articles will be published in the order of their approval for publication but the address of the retiring President and invitation articles will be published when received.

To comply with the International Rules of Botanical Nomenclature, Latin descriptions must be supplied to validate new species and genera.

Authors requiring reprints with or without covers should place an order for the copies wanted at the time of returning the proofs and they will be charged actual cost.

INDIAN PHYTOPATHOLOGICAL SOCIETY

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